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Sand Flies
(Diptera: Psychodidae: Phlebotominae): Significance, Surveillance, and Control in Contingency Operations

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AFPMB Technical Guides

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SECTION 1. INTRODUCTION

Sand flies belong to the subfamily Phlebotominae of the dipteran family Psychodidae. Sand flies are small, with a body about 3mm in length being typical for many species. They are noted for hairy bodies and wings and relatively long legs. At rest, the wings are held nearly erect and in a characteristic upright V-formation, unlike most other biting flies. Sand flies have nearly silent flight (Killick-Kendrick 1999, Maroli et al. 2012), presumably from the presence of hairs on their wings, which in combination with their small size, may explain their ability to inflict multiple bites on humans and remain undetected. Only females are able to pierce the skin of their vertebrate hosts to imbibe blood. Although not always felt, sand fly bites usually produce small, round, reddish bumps that may start itching hours or days later. Some species are selective in their feeding habits, but others feed on any suitable animal they encounter. They use their mouthparts to lacerate the host's skin, disrupting cells and causing a blood pool to form, from which they obtain their blood meal. Like most blood-feeding ectoparasites, they inject salivary proteins and other biochemicals into the bite wound, which then serve to inhibit blood clotting and promote blood flow.

In addition to being a tremendous nuisance when their biting interferes with and degrades mission performance, sand flies have been implicated as vectors of several disease agents, including those responsible for the various forms of leishmaniasis. Sand flies have been, and will continue to be, a major preventive medicine issue during military exercises and operations conducted within their geographic range. For these reasons, sand fly surveillance and control are key responsibilities of utmost importance for preventive medicine personnel. Control of sand flies can be difficult because immature habitats are poorly understood, which makes them difficult to target with available control measures. Moreover, resting adults often take refuge in inaccessible areas, such as small cracks in stone walls and behind tree bark. In addition, the behavior patterns of adults of different sand fly species are highly diverse, requiring tailor-made control solutions based upon a profound knowledge of their species-specific biology.

For many years, sand fly-borne pathogens occurred sporadically and at low levels. This appearance of control was a collateral effect of indoor residual spraying of houses with DDT for malaria control. Discontinuation of DDT use, along with other changes in human activity, have resulted in an increasing number of cases of leishmaniasis over the last 40 years. Additionally, the shift from military training operations in Panama and other areas in the Americas to military combat operations in Southwest Asia has changed the *Leishmania* species of interest and the incidence of sand fly-related diseases.

This TG provides basic information about the biology, surveillance and control of sand flies. Their medical importance and nuisance impacts are presented within the context of military operations and exercises. In accordance with Department of Defense policy on pesticide use, this TG also provides detailed guidance on surveillance and control strategies.

SECTION 2. SIGNIFICANCE OF SAND FLIES TO MILITARY OPERATIONS

2-1. Medical Impact of Sand Flies

Sand flies are a significant public health concern in many parts of the world, where they are known to transmit the agents of several zoonotic diseases to humans (Killick-Kendrick 1999, Ready 2013). Of the approximately 800+ described sand fly species in the subfamily Phlebotominae of the insect family Psychodidae (Order: Diptera), roughly 98 species are proven or suspected vectors of human leishmaniasis, including 42 *Phlebotomus* species in the Old World and 56 *Lutzomyia* species in the New World (Maroli et al. 2012). In addition to serving as vectors of the agents that cause cutaneous and visceral leishmaniasis (*Leishmania* spp.) (Killick-Kendrick, 1990), sand flies have been implicated as vectors of several arboviruses, including those that cause vesicular stomatitis and sand fly fever (Tesh, 1988), and members of the genus *Phlebovirus* (family Bunyaviridae), Chandipura (genus *Vesiculovirus*, family Rhabdoviridae) and Changuinola (genus *Orbivirus*, family Reoviridae) (Depaquit et al., 2010, Plyusnin et al. 2012). Sand flies are also known to transmit the bacterium *Bartonella bacilliformis*, which causes human bartonellosis (Carrion's disease) (Alexander, 1995). Leishmaniasis is found in about 90 countries (Lobo et al. 2011, Hotez et al. 2012) in Africa, South and Central America, South and Central Asia, the Mediterranean, and the Middle East, as well as in parts of the American states of Texas and Oklahoma.

The World Health Organization (WHO) estimates that roughly 350 million people worldwide are at risk of acquiring leishmaniasis, and that 12 million cases (including 60,000 deaths) occur annually. A small number (≈70) of locally acquired cases have occurred in the United States over the past 100 years. Although phlebotomine sand flies are known to occur throughout much of the eastern United States, the National Center for Medical Intelligence made an assessment that the risk of acquiring leishmaniasis in the United States is quite low and widespread leishmaniasis transmission is very unlikely. According to the WHO, leishmaniasis remains among the top emergent infectious diseases in the world, despite control measures. The number of cases is increasing primarily in the developing world due to human encroachment into the habitats of animal reservoirs and corresponding exposure to infected vectors, partly as a result of poor housing. The range of leishmaniasis in southern Europe and the United States has been expanding, and autochthonous cases of leishmaniasis have recently been documented outside their known historic range. These range expansions have been attributed to climate change (Fischer et al. 2011). Ecological niche modelling studies indicate that continued landscape modification and future climate change will heighten opportunities for the geographic expansion of vectors and leishmaniasis into North America from more southern latitudes (Moo-Llanes et al. 2013).

Cutaneous leishmaniasis (CL) is the most common form of the disease and is caused by a number of different *Leishmania* species in different parts of the world. It is characterized by a skin ulcer at the site of the sand fly bite that may persist for months before healing on its own (Fig. 2-1). Even without treatment, CL rarely causes severe illness but scarring from the lesions can be disfiguring. About 90 percent of the world's CL cases occur in Southwest Asia and South America. Mucocutaneous leishmaniasis (MCL), a less common form of the disease, occurs almost exclusively in South America. Infection by MCL normally involves the mouth and nose and can result in extreme disfigurement (Fig. 2-2). Diffuse cutaneous leishmaniasis (DCL) is the least common form of the cutaneous group and is generally associated with immunologically impaired persons (Fig. 2-3).

Fig. 2-1. Cutaneous leishmaniasis lesions on U.S. soldiers in Iraq. Photo: LTC Peter Weina, USA.

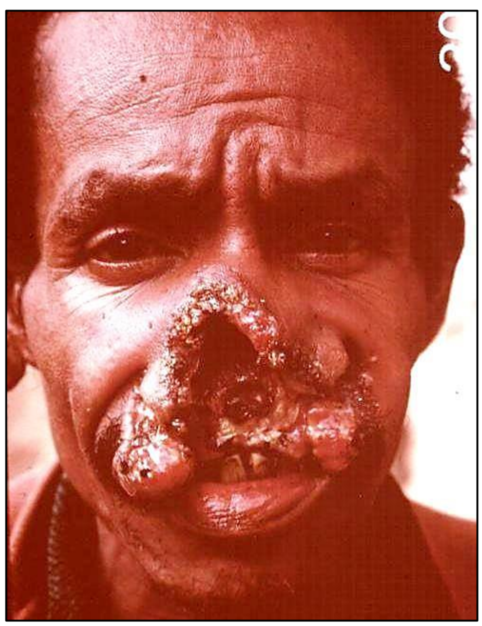


Fig. 2-2. Mucocutaneous leishmaniasis lesion. Photo: source unknown.

Fig. 2-3. Diffuse cutaneous leishmaniasis. Photo: from Chaudhary et al. (2008).

Visceral leishmaniasis (VL), the other major form of the disease, is caused by species different from those responsible for CL. The parasite species involved include *Leishmania donovani* and *L. infantum/L. chagasi*. VL affects the internal organs of the body, including the spleen, liver, and bone marrow (Fig. 2-4). VL is the most severe form of leishmaniasis and is usually fatal if left untreated. More than 90 percent of the world's reported VL cases occur in Bangladesh, India, Nepal, Sudan, and Brazil (Mondal et al. 2009, Bern et al. 2010).

Fig. 2-4. Visceral leishmaniasis. Photo: World Health Organization.

Post-kala-azar dermal leishmaniasis (PKDL) is a complication of VL in areas where *Leishmania donovani* is endemic. PKDL is characterized by a prominent rash, usually in patients who have recovered from VL (Fig. 2-5), which may appear 6 months or later following treatment for VL. However, it may also occur earlier or even concurrently with VL. Although PKDL generally heals spontaneously in the majority of African cases, it rarely heals spontaneously among patients in India. PKDL may play an important role in maintaining and contributing to transmission of VL.

Fig. 2-5. Post-kala-azar dermal leishmaniasis. Photo: World Health Organization.

In the Old World, most leishmaniasis foci are found in dry, semi-arid habitats. By comparison, CL in the New World is primarily associated with forested areas, the exception being the foci in the United States (Texas and Oklahoma) and northern Mexico that are found in semi-arid areas. New World VL is generally associated with a peri-urban environment (Fig. 2-6).

Leishmania parasites are maintained mostly in animal reservoirs (Killick-Kendrick 1999, Membrive et al. 2012, Moncaz et al. 2012), including dogs, cats, rodents, hyraxes (small, hoofed mammals native to Africa), and marsupials. Infected dogs, foxes and small rodents usually show lesions caused by leishmanias (Fig. 2-7), but other animal reservoirs of the parasites usually have no overt signs of this infection. There are foci where no non-human reservoir has been found despite intense searches, lending credence to a solely human-sand fly cycle (i.e., anthroponotic). Typically, parasites are transmitted to humans through the bite of an infected female sand fly, although humans can be infected through blood transfusion in rare cases. There also have been rare cases of transplacental and venereal transmission. Direct human-to-human transmission does not occur. Leishmaniasis distribution is often highly focal and dependent on sand fly habitat. Vector-borne transmission to animals and humans typically occurs only in areas close to sand fly habitats, including stone walls or buildings, animal harborage such as rodent burrows, and waste material accumulation points. In part this can be explained by the short hopping flights of sand flies, observed by researchers and hypothesized to be indicative of poor flight ability. However, this flight behavior may simply be a response to the fact that appropriate hosts themselves are often not far from these habitats. Some species of sand flies are fast and agile flyers that may range far from these kinds of habitats to contact vertebrate hosts opportunistically (Killick-Kendrick 1999). Other sand fly species are weak flyers that may not be detected by surveillance or cause bite complaints at higher wind speeds.

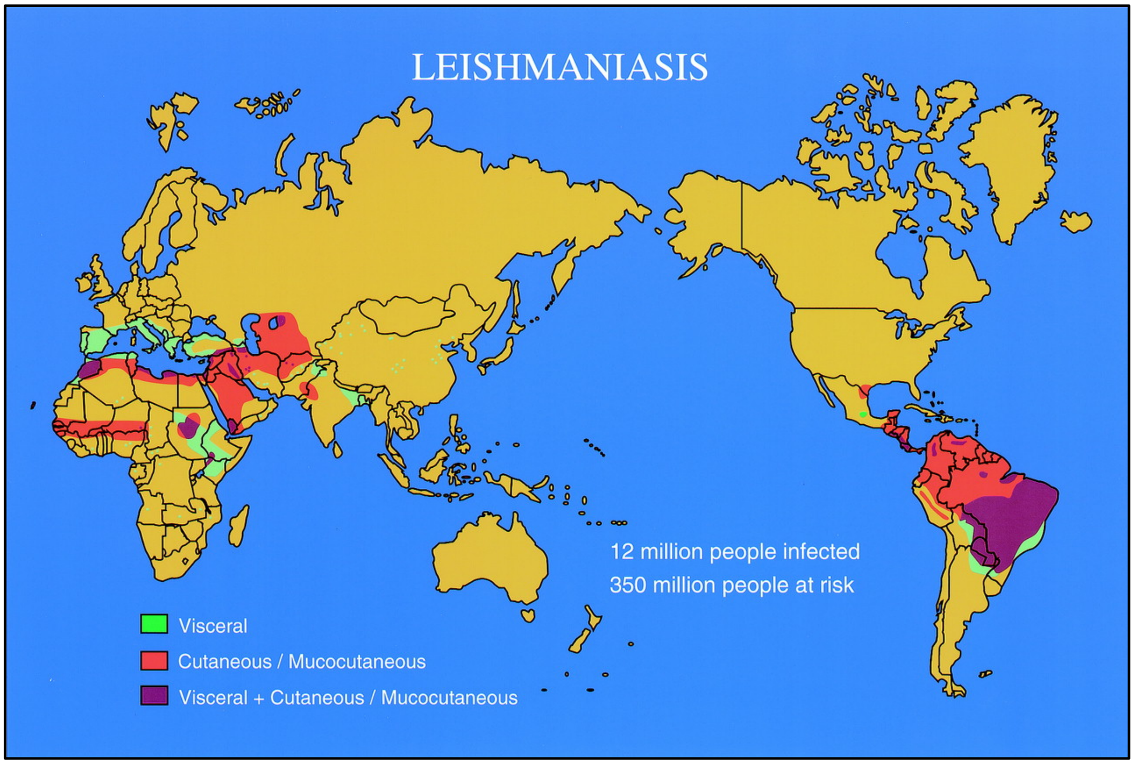


Fig. 2-6. Distribution of leishmaniasis worldwide. Photo: Clinical Microbiology Reviews, American Society of Microbiology, approved for educational use.

Fig. 2-7. A dog with visceral leishmaniasis. Note lesion on nose. Photo: Wikipedia, used with permission.

Because of the risk of disease transmission, especially leishmaniasis, and annoyance or distraction from their bites (Fig. 2-8), sand flies may interfere with military operations (Coleman et al. 2006, 2007, Faulde et al. 2009, Kroger et al. 2011). Indeed, sand flies historically have had, and continue to have, an impact on military and disaster relief operations, and refugee health support operations, underscoring the enduring requirement for sand fly surveillance and control.

Fig. 2-8. Torso of a U.S. soldier in Iraq showing many sand fly bite marks. Photo: Lt Col Doug Burkett, USAF.

Fig. 2-9. Child with *Verruga peruana* infection. Photo: Wikipedia, public domain.

Bartonellosis

Lutzomyia verrucarum is the suspected vector of the bacterium *Bartonella bacilliformis*, which causes Carrión's disease (Oroya fever, *verruga peruana*). This infectious disease is rare, being found only at higher elevations in Andean regions of Peru, Ecuador, and Colombia. Infection may result in two clinical phases: an acute hemolytic phase (Oroya fever) and a chronic eruptive phase associated with skin lesions (*verruga peruana*) (Fig. 2-9). The acute phase is a sudden, potentially life-threatening infection associated with high fever, decreased levels of circulating red blood cells, and transient immunosuppression. This stage may last from 2 to 4 weeks. The associated death rate can be up to 90% but is usually less with timely antibiotic therapy. Neurological complications can occur in about 20% of patients and may include altered mental state, agitation, involuntary muscle movement, coma, meningitis, and paralysis. Secondary bacterial infections may be an additional complicating factor. The chronic phase of the disease, *verruga peruana* (Peruvian wart), consists of a benign dermal eruption resulting in a reddish-purple welt or nodule on the skin. It may appear up to 2 months after the patient has recovered from Oroya fever.

Sand Fly Fevers

Sand fly fevers (also known as Phlebotomus or Pappataci fevers) are febrile viral (Bunyaviridae, *Phlebovirus*) infections transmitted by sand flies in the genus *Phlebotomus*. They occur in the subtropical regions of the Eastern Hemisphere, particularly in southern Europe (Depaquit et al. 2010) and the eastern Mediterranean, North Africa, the Balkans, Southwest Asia, and India (Alkan et al. 2013). Infections may range from mild febrile illness to severe central nervous system complications. Although incapacitating, sand fly fevers have negligible associated mortality, and full recovery may take several weeks. Additional sand fly fever viruses are endemic to the New World, but their distribution is extremely spotty and their symptoms less well known than those of the Mediterranean forms.

Vesicular Stomatitis

Vesicular stomatitis is a viral (Rhabdoviridae, *Vesiculovirus*) zoonotic disease of domestic livestock and wild animals, but it can lead to a relatively rare flu-like illness in humans. Human mortality from vesicular stomatitis is negligible. The widely distributed sand fly *Lutzomyia shannoni* is a known vector of vesicular stomatitis virus.

2-2. Sand Flies as Nuisance Pests

In the absence of potential disease transmission, sand fly bites can be painful and result in dermal reactions that may become secondarily infected. When sand fly densities are high, and deployed military personnel lack or do not use proper personal protective measures, large numbers of bites can be psychologically demoralizing and result in distracted or poor duty performance. Unprotected personnel may receive multiple bites while sleeping, especially in locations without air conditioning, causing them to infer that they were attacked by other arthropods (e.g., bed bugs, scabies, lice), which may frustrate preventive medicine team resources.

SECTION 3. IMPORTANT SAND FLY SPECIES: BIOLOGY AND BEHAVIOR

3-1. General

Sand flies occur primarily in the tropics and subtropics, with only a few species ranging into the temperate zones of the northern and southern hemispheres. They are limited to areas with temperatures above 60^{o}F for at least 3 months of the year. Sand flies can be very difficult to identify to species using morphological characters. In general, the male characteristics used for species identification are more apparent than those used for female identification. However, identification of adult female sand flies remains critical because they are the gender capable of transmitting disease to humans. In areas of the tropics with numerous endemic species, a sand fly specialist is usually needed to distinguish among species (contact Walter Reed Biosystematics Unit or local experts where available).

3-2. Life Cycle

Most of our knowledge about the life cycle of various sand fly species comes from laboratory-reared specimens. Sand flies have an extended, complete life cycle (egg, larva, pupa, adult), which generally lasts from 1 to 3 months. In temperate climates, overwintering in one of the life stages may further extend the life span.

Immature Stages. Eggs are dark and elliptical in shape. They are laid individually in small batches in protected areas with high humidity and sufficient organic matter to serve as food for the larvae (Feliciangeli 2004). Under optimal conditions, eggs typically hatch in 1-2 weeks. The larvae are small and caterpillar-like with a well-developed head capsule and numerous brush-like or spatulate setae on the body, and long caudal setae that are nearly as long as the body. The first instar larvae have an egg-bursting spine on the head capsule and 1 pair of caudal setae. The 2nd, 3rd and 4th larval instars have 2 pairs of caudal setae, but otherwise, except for size, appear similar to the 1st instar larvae (Fig. 3-1). In the 4th instar, larvae bear a darker sclerotized plate on the dorsum of the last abdominal segment. Development time for some sand fly larvae may be as short as 18 days, but it may be prolonged to months during cold weather in temperate zones. Prior to transforming to a pupa, the mature 4th instar larva ceases to feed and seeks a place to pupate that is usually drier and perhaps more protected and anchors itself to a surface, such as a leaf or stone. Sand fly pupae may be distinguished by the cast off skin of the 4th instar larva, including the caudal setae, still attached to the distal end of the body (Fig. 3-2). The pupal stage usually lasts 7-12 days; males usually emerge before females.

Fig. 3-1. Fourth instar larva of *Lutzomyia shannoni* Dyar. Photo: Jerry Butler, University of Florida.

Fig. 3-2. Pupa of *Lutzomyia shannoni* Dyar. Photo: Jerry Butler, University of Florida.

Adults. Within 24 hours of emergence, male sand flies rotate their external genitalia 180º and become sexually mature (Fig. 3-3). Males may be aided in locating females through the use of pheromones (male or female), or by finding a resting site or vertebrate host where females are present. In the latter case, mating sometimes occurs while females are blood feeding (Fig. 3-4). Premating courtship behavior, including rapid male wing beating, has been witnessed in several species. Mating swarms of some sand flies (*Lutzomyia longipalpis, Phlebotomus argentipes*) have been reported as "lekking behavior" (Jones et al. 2000) involving aggregations on hosts or near them, but swarming flights of male sand flies have not been observed like those of midges and mosquitoes.

Adult phlebotomine sand flies can be confused with harmless drain flies (Fig. 3-5), which also belong to the family Psychodidae, and adult female mosquitoes (Fig. 3-6). The following series of photos helps distinguish the differences between sand flies (Fig. 3-7) and these other flies.

Fig. 3-3. Male sand fly, *Phlebotomus* sp. Photo: U.S. Centers for Disease Control and Prevention.

Fig. 3-4. Female sand fly, *Phlebotomus papatasi*. Photo: Frank Collins, CDC.

Fig. 3-5. Drain fly (Psychodinae) (top) and sand fly (Phlebotominae) (below) collected in southern Iraq, 2010. Photo: Seth Britch, USDA.

Fig. 3-6. Female sand fly (above) and female mosquito (below) collected in southern Iraq, 2010. Photo: Seth Britch, USDA.

Fig. 3-7. *Lutzomyia* female sand fly (left) and male sand fly (right). Photos. Pete Perkins.

3-3. Feeding Behavior

Both adult males and females require a source of carbohydrates for energy. The source of sugar remains a mystery for many species; however, biochemical analysis has provided evidence of feeding on honeydew from aphids and other homopterans, nectar from flowers and fruits, and other plant juices. This behavior has led to the development of toxic sugar bait as a control method. Carbohydrates may also affect the development and infectivity of *Leishmania* in sand flies. Autogenous females may be able to lay one small batch of eggs without first feeding on blood, but a blood meal is required for subsequent egg batches. Anautogenous females require vertebrate blood for the maturation of their eggs. Some species feed only once between successive ovipositions, whereas others may take multiple blood meals during a single oviposition cycle. Host choices vary between species; some feed almost exclusively on a small array of vertebrate species, but others are more opportunistic in their choices.

Most human-biters feed at dusk, during the evening and throughout the night, but others will also attack in daytime if disturbed in their daytime resting sites. Windless or fairly calm conditions, along with other optimal circumstances, or calm conditions following a period of turbulent/windy weather may suddenly induce greater-than-expected numbers of sand flies to seek hosts (Colacicco-Mayhugh et al. 2011). Most anthropophilic sand flies are exophilic, biting persons outside of houses, tents or other habitations. Some others readily enter human dwellings or field structures, such as bunkers, to bite occupants day or night.

3-4. Flight Range

Few studies have been successful in determining maximum flight range of sand flies, although distances up to 2 km in more open terrain are often cited. In thick forest and jungle areas, 100-200 m is probably the maximum. In open areas most flight is assumed to be close to the ground where wind velocity is the lowest; however, in the presence of human hosts sand flies may be observed in flight at any convenient height to reach exposed skin. Vertical movement, perhaps daily, has been shown for some species from forest floor to canopy, or higher if sand flies are taken up by air currents.

3-5. Resting Sites

Sand flies use resting sites while waiting for dusk and seeking hosts or mates. Blood-fed females also rest while waiting for their blood meal to digest and their eggs to mature. The types of resting sites used by adult sand flies may vary according to season, microhabitat, amount of moisture, and sand fly species. Sand flies may abandon resting sites that become too wet but then return to them as the sites dry out. Tropical rain forests offer the greatest variety of sites. In the Americas, the forest floor itself is the most extensive microhabitat used by resting sand flies (Memmott 1991). Dry tree holes or hollows and tree buttresses are commonly used in forested areas. High crowns of palm trees may harbor sand flies (Poche et al. 2012). Caves, rocky outcroppings, rock walls, termite mounds and animal burrows are especially useful in drier areas. Some endophilic species that enter homes to feed may rest on the inside walls for a short time, usually leaving within 24 hours after the blood meal.

3-6. Sand flies of the Western Hemisphere

Three genera of sand flies are currently recognized in the Western Hemisphere, *Brumptomyia*, *Warileya* and *Lutzomyia*, of which only the last contains species of medical significance (Oliveira et al. 2012). Multi-entry computerized keys containing high-resolution photographs are available for determining both males and females to genera, subgenera, and species within 11 subgeneric groups at http://wrbu.org/southcom_SFkeys.html. For a list of genera-specific characters, see http://www.wrbu.org/sf_gnra.html. A succinct web-linked summary of CENTCOM sand fly morpho-characters and keys is available at: http://www.syriaproject.info/#!sandfly-morpho-tools/c1jxy

Genus *Brumptomyia* Franca and Parrot, 1921

This genus includes at least 22 species that are distributed from southern Mexico to northern Argentina. Adults are commonly found in armadillo burrows, sometimes on tree trunks, or in light and flight traps. None of the species has been reported biting humans. Identification of species is based almost entirely on male structures.

Medical Importance: None known.

Genus *Warileya* Hertig, 1948

This genus is represented by 6 species occurring in Costa Rica, Panama, Colombia, Peru, and Bolivia. Some species are known to be anthropophilic (man-biting), but none have been incriminated as vectors of human pathogens.

Medical Importance: None known.

Genus *Lutzomyia* Franca, 1924

Some 382 species of *Lutzomyia* have been described from the Americas (Young and Arias 1991, Young and Duncan 1994), ranging from southern Canada to northern Argentina. A Brazilian research group has published an alternative taxonomic scheme, which elevates many subgenera to generic level (Galati 1995, 2003). This has not been universally accepted, so for this TG the genus *Lutzomyia* will be discussed in its classic sense. Over 31 species of *Lutzomyia* have been implicated as proven or suspected vectors of *Leishmania* spp. to reservoir hosts or humans, with several species proven to transmit *Bartonella* bacteria and numerous arboviruses. Examples of some of these follow. For further information, the full list of implicated vectors and links to original taxonomic descriptions, refer to the Walter Reed Biosystematics Unit's Sand Flies of Medical Importance at http://wrbu.org/southcom_SF.html.

***Lutzomyia anthophora* (Addis, 1945)**

Distribution: Mexico and southwestern United States.

Bionomics: This species has been implicated as the vector involved in the transmission of enzootic *Leishmania mexicana* and Rio Grande virus in southern Texas (Young and Duncan, 1994). It is principally found in southern plains woodrat nests, generally with little movement out of the protected nest environment. The entire sand fly life cycle is thought to occur in the nest area. It is not known if *Lu. anthophora* is responsible for transmission of this parasite to people in the endemic area of Texas and northern Mexico.

***Lutzomyia diabolica* (Hall, 1936)**

Distribution: Mexico and Texas.

Bionomics: This species is an aggressive biter that often attacks personnel in military training areas in Texas during summertime, and is competent to transmit *L. mexicana* but has not been found naturally infected (Calborn et al. 2009). However, it has been implicated as a vector involved in zoonotic transmission of *Leishmania mexicana* in Texas based on its distribution in *Le. mexicana* foci, its man-biting behavior and proven vector competence (Lawyer et al. 1987, Lawyer and Young 1987). Mainly active nocturnally, they sometimes bite during daytime and have been found resting on walls inside latrines (Young and Perkins 1984).

***Lutzomyia longipalpis* (Lutz and Neiva, 1912)**

Distribution: Argentina, Bolivia, Brazil, Colombia, Costa Rica, El Salvador, Guatemala, Honduras, Mexico, Nicaragua, Panama, Paraguay, Venezuela.

Bionomics: *Lutzomyia longipalpis* is undoubtedly the best-studied sand fly in the Americas. There are many laboratory colonies, which have provided material for studies on taxonomy, physiology, biology, behavior, and host/parasite relationships of this species. There is mounting evidence that *Lu. longipalpis* represents a complex of multiple sibling species (not interbreeding) with contrasting behaviors, genetics and ecology. Some species of the *Lu. longipalpis* complex are the principal vectors of *Leishmania infantum chagasi*, the etiologic agent of VL in the Americas. *Lutzomyia longipalpis* is geographically widespread and locally abundant, especially on farms where domestic animals are kept. Generally it is found more abundantly in the peri-urban environment than in forested areas nearby (de Melo Ximenes et al. 2006, Casanova et al., 2013). *Lutzomyia longipalpis* is an opportunistic feeder on mammals. The natural cycle for VL includes this species and either wild or domestic canids (dogs or foxes). The close proximity of a domestic reservoir host, a competent vector, and humans creates an ideal environment for transmission of *L. infantum chagsi* to man. Sand fly populations vary throughout the year, with the highest populations encountered toward the end of the rainy season in many parts of Central and South America.

***Lutzomyia shannoni* (Dyer, 1945)**

Distribution: Argentina to United States.

Bionomics: This species has the widest geographical range among sand flies in the Americas. In the U.S., it has been recovered as far north as New Jersey. In the laboratory it has been shown to support the growth of Old World *Leishmania major* (Claborn et al. 2009). However, transmission has not been conclusively demonstrated, and it is not thought that a domestic transmission cycle involving infected military members returning from *L. major* endemic areas of Southwest Asia is likely to be established in the U.S. In the U.S., *Lu. shannoni* has rarely been found biting humans, but elsewhere in its range it may be much more abundant and highly anthropophilic. It has been shown to transmit vesicular stomatitis virus in southeastern coastal regions of the U.S (Young and Duncan, 1994).

***Lutzomyia verrucarum* (Townsend, 1913)**

Distribution: Colombia, Peru.

Bionomics: This species is the suspected vector of the etiologic agent of Carrión's disease and leishmaniasis in Peru, where it has been found at elevations up to 3,300 m above sea level. In Colombia, it is a suspected vector of *L. braziliensis* (Young and Duncan, 1994). It feeds on many species of mammals at higher altitudes in the Andes Mountains. This species has been colonized in the laboratory, but requires a cooler rearing temperature than most species for optimum growth (21°C).

***Lutzomyia whitmani* (Antunes and Coutinho, 1939)**

Distribution: Argentina, Brazil, French Guiana, Paraguay, Peru.

Bionomics: One of many species responsible for the transmission of the *L. braziliensis* (CL and MCL) complex in tropical rainforests, *Lu.whitmani* has also been implicated in the transmission of *L. guyanensis* and *L. shawi* in Brazil. This species readily moves from the canopy, where it feeds on sloths (reservoir host), to the forest floor, where it aggressively feeds on humans.

3-7. Sand flies of the Eastern Hemisphere

Sand flies in the Old World (Africa, Asia, Europe, and Australia) tend to be more subtropical in their distribution compared to the predominantly tropical species in the Americas. Relatively few species occur south of the Sahara. Their habitats tend to be drier, though they occur at altitudes below sea level (Dead Sea) to over 3,000 m (lower Himalayas). In many arid or semi-arid habitats, sand fly populations are highest toward the end of the rainy season and lowest toward the end of the dry season. In hot, dry deserts or in dry temperate climates with hot summers and cold winters, adults of some species may disappear entirely during the driest and/or coldest seasons of the year. Three genera of sand flies are recognized in the Old World (Seccombe et al. 1993, Ready 2013): *Phlebotomus*, to which all of the medically important species belong; *Sergentomyia*, in which only a few species bite man; and *Chinius*, a small cave-dwelling genus with four described species. Further details on differentiation of these genera can be viewed at: http://www.wrbu.org/sf_gnra.html.

Genus *Chinius* Leng, 1987

Bionomics: A small group of only four cavernicolous (cave dwelling) species described from China, Laos, the Philippines, and Thailand. Very little is known of their biology, bionomics, or distribution.

Medical Importance: None known.

Genus *Sergentomyia*

Bionomics: This is a large genus with over 285 species. Females of a few species have been known to bite humans. Most species feed on reptiles, and some of these may be involved in the transmission of *Sauroleishmania*. The presence of *Leishmania*-like flagellates in captured sand flies may wrongly inflate infection rate calculations for the human-infecting *Leishmania* spp. Since *Sergentomyia* sand flies resemble and usually coexist with *Phlebotomus* species, collection numbers may give a false impression of the abundance of medically significant sand flies in a region.

Medical Importance: Little is known about the medical importance of this genus. However, *Sergentomyia dubia* Parrot, Mornet and Cadenat, 1945 was found infected with *L. major* in Senegal (Desjeux & Waroquy, 1981). Lawyer et al. (1990) found that *Leishmania major* developed slowly in *Sergentomyia schwetzi* and the parasites did not develop beyond "procyclic" promastigotes.

Genus *Phlebotomus*

Bionomics: Females of *Phlebotomus* obtain blood meals primarily from mammals. Many species have a very close affinity with their primary mammal hosts, immature stages being found in the organic matter of the host's burrow/nest and resting adults found almost exclusively in the burrows as well. Because they generally inhabit drier environments compared to those occurring in rainforests of the Americas, available breeding and resting sites for *Phlebotomus* species are more restricted. Nevertheless, the mass emergence of sand flies from such restricted habitats is a hallmark of some species. Extended periods of inclement weather and consequent limited feeding opportunities can be a catalyst for mass emergences.

Medical Importance: This genus contains all known medically important species in the Old World, including incriminated vectors of mammalian *Leishmania* species (*L. infantum*, *L. donovani*, *L. aethiopica*, *L. major*, and *L. tropica*) and of sand fly fever serogroup arboviruses, which seem to be restricted to mammalian vertebrate hosts. Sand fly bites can be extremely irritating, leading to urticarial rashes as well as secondary bacterial infections.

The following are representative species that are important vectors in all or parts of their range. For further information on the taxonomy, bionomics and biomedical importance of Old World sand flies by command (EUCOM, CENTCOM, AFRICOM, PACOM), please refer to the Walter Reed Biosystematics Unit's website on Sand Flies of Medical Importance at: http://www.wrbu.org/command_aors_SF.html.

***Phlebotomus argentipes* (Annandale and Brunetti, 1908)**

Distribution: Nepal, India, Sri Lanka and Pakistan eastward to Myanmar, Thailand, Vietnam, Laos and Indonesia.

Bionomics: This species is a competent vector of *Leishmania donovani*, the agent of classic VL. In parts of India, humans are the only known vertebrate reservoirs of this pathogen. Its preferred hosts are cattle and humans and it readily enters houses and other habitations. Larvae can be common in soil samples in the earth floors of human habitations and cattle shelters (Ozbel et al. 2011).

***Phlebotomus duboscqi* Neveu-Lemaire, 1906**

Distribution: Burkino Faso, Ethiopia, Gambia, Ghana, Kenya, Mali, Mauritania, Niger, Nigeria, Senegal, Sudan, Togo, and Yemen.

Bionomics: A proven or suspected vector of *L. major* throughout its range (Lawyer et al. 1990), this species is generally associated with rodent burrows that provide for the enzootic cycle. Hanafi et al. (2012) showed that *P. duboscqi* could acquire, develop and transmit *Leishmania tropica* under experimental conditions. It is an opportunistic feeder and more commonly found in sylvatic rather than peridomestic or domestic environments (Fryauff et al. 1995, Senghor et al. 2011). However, in Mali this species is found almost exclusively in periodomestic/domestic environments.

***Phlebotomus martini* Parrot, 1936**

Distribution: Horn of Africa - Ethiopia, Kenya, Sudan, Somalia, Uganda.

Bionomics: A proven vector of *L. donovani* in Ethiopia and Kenya, and a suspected vector in Somalia and Uganda. Habitat is desert vegetation associated with *Macrotermes* termite mounds, which are thought to provide ideal breeding and resting habitats. However, termite mounds are more widespread than both the vector and the disease.

***Phlebotomus orientalis* Parrot, 1936**

Distribution: Sahel region of Africa from Niger to Egypt; Ethiopia, Kenya, Rwanda, Uganda; Saudi Arabia and Yemen on the Arabian Peninsula.

Bionomics: A proven vector of *L. donovani* in Sudan, and a suspected vector in Chad, Ethiopia, Kenya, Saudi Arabia, and Yemen. This species is associated with *Acacia-Balanites* forests and black cotton clay soils that crack deeply during the dry season, providing microhabitats for the sand flies (Elnaiem 2011).

***Phlebotomus papatasi* Scopoli, 1786**

Distribution: Occurs in a broad swath from France across most of the Mediterranean Basin, and eastward to India, including the Arabian Peninsula and Ethiopia.

Bionomics: This species is an opportunistic feeder that can be highly anthropophilic when the situation permits. Females readily enter houses and other structures to bite humans. Also found abundantly associated with small rodent burrows in certain arid areas of its range. It is a proven vector of *L. major* (responsible for zoonotic CL) in many countries. Sand fly fever virus Naples and Sicilian forms (Pappataci fever) are often found in humans where only this sand fly occurs.

***Phlebotomus perniciosus* Newstead, 1911**

Distribution: Mediterranean Basin to Azerbaijan.

Bionomics: A proven vector of *L. infantum* (VL) and Toscana virus, the leading cause of acute meningitis between May and October in northern Mediterranean countries.

***Phlebotomus sergenti* Parrot, 1917**

Distribution: This is one of the most widely distributed sand fly species in the Old World, ranging from Portugal and the Mediterranean Basin eastward to Ethiopia, the Arabian Peninsula, and India.

Bionomics: *Phlebotomus sergenti* is the main vector of *L. tropica,* the agent of urban anthroponotic ("Aleppo boil") and occasionally zoonotic CL, in various parts of its range (Maroli et al. 2009, Orshan et al. 2010). It is considered to be highly anthropophilic but can be an opportunistic feeder. In much of its range, *P. sergenti* demonstrates bimodal population peaks in late spring and late summer. Females exhibit exophilic and exophagic behavior, which limits the effectiveness of indoor residual spray programs. However, use of insecticide-treated bed nets has been shown to be an effective method of reducing disease incidence. Breeding places are often associated with hyraxes in caves, as well as other animal burrows (Moncaz et al. 2012).

SECTION 4. SURVEILLANCE AND EVALUATION OF CONTROL EFFORTS

Prior to conducting surveillance for sand flies, AFPMB Technical Guide 48, Contingency Pest and Vector Surveillance, should be consulted for detailed background information on the rationale and approach for conducting entomological surveillance.

Selection of sand fly collection methods will depend on the objectives of the study, the degree of familiarity with the local fauna, and the ecology of the study site. If the intent is to collect live sand flies for colonization or experimentation, specific techniques will be required. For locations where little is known about sand fly populations and their habitats, a combination of sampling methods should be used in order to determine the species spectrum. If the habits and ecology of the target sand fly population are well known, one or two methods may suffice, particularly if they have previously proven successful.

The first step in undertaking a surveillance program in an operational setting is to examine area maps to determine the topographical and land use features that might offer potential breeding sources and serve as likely sites for surveillance. The WRBU website VectorMap (http://www.vectormap.org/) is an excellent source for such information, and serves as the DoD central repository for sand fly distribution and sand fly incrimination data. Basic ecological and meteorological knowledge of the geographical location will increase the success of surveillance efforts. Ideally, surveillance sites should be located between local populated areas or other potential disease sources in populated regions or between the populated areas and vector breeding sources, if known. After potential breeding sources have been identified and traps have been set, their locations and positions, ideally using Military Grid Reference System (MGRS) coordinates, should be recorded in VectorMap as a permanent record of the surveillance program. To the extent possible, identifying surveillance data collection sites should be standardized. One option for ensuring standardization is to use reference data collection forms developed by WRBU.

4-1. Necessity of Sand Fly Surveys

Sand fly breeding areas may be off the installation and difficult to locate, placing larval control beyond the charge of preventive medicine/public health assets, and making it necessary to concentrate on surveillance and control of adults. Sand fly surveys provide a sound basis for recommending and implementing control measures. Sand fly surveys are also necessary to track population trends and evaluate the effectiveness of control measures. Larval surveys are extremely labor intensive and rarely time/cost effective in determining sand fly populations; however, knowledge of potential breeding sites can assist in the siting of adult surveillance efforts. Larval habitats can be considered as domestic, peridomestic, or sylvatic (Feliciangeli 2004) (Table 4-1).

4-2. Five Elements of Effective Sand Fly Surveillance

a. Surveillance to identify the presence, species, and estimate size of sand fly populations.
b. Sustained monitoring of populations and environmental conditions that favor

breeding.

c. Evaluation and interpretation of survey results.
d. Initiation of control measures when established thresholds have been exceeded and notification of appropriate units responsible for conducting control measures.
e. Continued surveillance to determine the success of control measures.

4-3. Surveillance Program SOPs

Surveillance programs should be documented in Standard Operating Procedures (SOPs) or protocols that are region specific. Information should include:

a. Who will do the surveillance? Specify the responsible units.
b. How will the surveillance be conducted? List and describe the techniques and procedures that will be used.
c. Where are the surveillance locations? Clearly identify all locations on a map with GPS coordinates (if possible).
d. When will the surveillance be conducted? Include the rationale for the frequency of surveillance and when complaints are evaluated and addressed.
e. What are the criteria for initiating control measures? Identify the thresholds to be used and the recommended control measures.

Table 4-1. Breeding sites and larval habitats for sand flies.

Domestic sites:

Abandoned buildings	Basements and cellars of houses
Cracks in mud floors and walls	Soil in human dwellings

Peridomestic sites:

Animal burrows	Animal shelters (cattle, pigs)	Caves
Chicken coops	Soil at the base of old walls	Dry excreta of small domestic animals
Earth dikes	Embankments	Latrines
Rotted manure	Rubbish in the street	Under stones
Debris and soil cracks	Wells	

Sylvatic sites:

Ant nests	Termite mounds	Burrows of small rodents & other animals
Dry cesspits	Drains	Garbage
Hollow trees	Leaf litter on forest floor	Nests of terrestrial tortoises
Bird nests	Rocks, between and under	Roots of large trees
Soils at base of trees	Soil under overhanging rocks	Caves

4-4. Threshold Values

An effective surveillance program must have a way to determine the basis for control measures. The mere presence of sand flies does not automatically initiate a recommendation for control measures to be implemented. Thresholds are established to help predict when control measures are needed. The threshold value itself is an index calculated from surveillance data. Continuous surveillance over an extended period of time may be required to establish reliable threshold values. Long-term surveillance data may also reveal identifiable trends that will protect personnel by allowing control measures to be initiated just before a serious problem occurs (Faulde et al. 2008, 2009, Tiwary et al. 2013). Threshold values will vary at different geographical locations depending on such factors as species, area involved, habitat, collection technique, number of complaints, and disease potential. In certain regions, such as developing nations in the tropics, sand fly problems may be readily apparent in the absence of surveillance. Regardless, sand fly surveillance is necessary to determine the effectiveness of control measures and to identify seasonal fluctuations and temporal population trends. Thresholds are only indicators and therefore should not be the only factor used in the decision to recommend control measures.

4-5. Initiating Sand Fly Surveillance

a. Develop lists of all sites where adult flies could be problematic and where immature stages might develop.

If feasible, surveillance at off-installation locations may be necessary to fully describe the scope of the problem and facilitate effective control measures.

b. Conduct a preliminary survey at all potential sand fly infestation sites.

The purpose of this survey is to identify the areas with existing sand fly problems.

c. Contact units or activities that are (or should be) concerned with sand fly control.

In some instances, units may not be aware that sand flies are the source of nuisance problems, which may require educational briefings to resolve. All personnel must be aware of the objectives of the surveillance program and their role in it. Meet with the appropriate units/activities to discuss:

(1) What actions are being accomplished for sand fly control?
(2) How can control actions from various organizations be integrated?
(3) What criteria are used to initiate control measures, such as:
- surveillance data?
- complaints?

(4) Whether previous problems related to sand fly control have been identified.
(5) What surveillance and control equipment, procedures, and activities will look like; this will increase acceptance and participation by the local unit, reduce the likelihood of interference with or damage to equipment, and increase success of the program.

d. Initiate sand fly surveillance that consists of surveys for:

(1) presence and numbers of flies.
(2) favorable larval development and/or adult resting sites.

e. Develop thresholds for control and a policy for dealing with complaints.

(1) Document all aspects of sand fly surveillance. All procedures should be written as an SOP to ensure consistency in data collection among personnel and over time. Include maps to show sand fly sampling sites, using GPS to accurately identify sampling site locations, and supplement each set of coordinates with a short written description of the site and where the trap should be placed, as well as any key information, such as a local point of contact, to reduce the likelihood the trap will be tampered with or moved.

(2) Estimating population density for a vector or pest is the most common use of arthropod sampling data after the determination of their presence or absence in the area of operations. For military deployments, measures of relative abundance of vector/pest populations are the most practical means of assessing their size. Sampling for obtaining an estimate of population density can be most easily achieved through a predetermined systematic approach. For example, systematic sampling may involve placing light traps at the same locations, at the same height from the ground, and for the same length of time. A similar approach is to develop a serial (or longitudinal) sampling method where trapping is conducted at the same locations, but at different times or dates to account for seasonality of the vectors.

(3) The actual number of samples to be taken depends on the personnel available to conduct the work and the complexity of the area of operations and associated vector/pest habitats. However, surveillance should always strive to use a minimum of three (3) concurrent samples of each type in an immediate sampling area to account for natural variation. For example, use three CDC traps in the same immediate area, if possible. Sampling should also be accomplished on a scheduled basis, when possible, to account for weather-related changes and seasonality. The extent and location of all continued sampling should be based on sound baseline information determined at the outset of the deployment. On the other hand, a newly arriving unit should not assume that prior surveillance and control activities are optimal. Camp configurations, landscape patterns, and natural populations change constantly, and the newly arrived preventive medicine leader should scrutinize all surveillance and control SOPs, including trap locations, to evaluate whether such SOPs adequately reflect current observed conditions.

(4) In order to implement control or management decisions, an action threshold must be established based on surveillance data. Although baseline surveillance provides the important initial information on presence or absence of a vector or pest, abundance data collected during additional surveillance can provide important insights concerning relative population dynamics. For example, if the number of female sand flies increases beyond a pre-established abundance index, then control measures may be warranted. The action threshold will be unique for different operational locations. However, if sand fly populations are large and represent a continuing threat to force health, establishment of an action threshold may be irrelevant. Calculation of an

abundance index is the foundation for determining an action threshold. For example, a weekly abundance, or trap index (TI), for adult female sand flies collected in CDC traps can be calculated as:

$$\textbf{TI=} \quad \frac{\textbf{Total female sand flies trapped}}{\textbf{Total trap nights}}$$

Where total trap nights is calculated by multiplying the number of traps used by the number of nights operated.

This index of abundance can be modified as appropriate for particular species of sand flies, and can also be adapted to evaluate sand fly population dynamics among different days, especially following control measures to determine if they were effective.

4-6. Sampling Methods and Surveillance Data

a. Direct searches

<u>Larvae</u>

Searches for the immature stages of sand flies should be directed to protected habitats with high humidity and soil moisture and an abundance of decomposing organic matter, such as leaf litter or animal feces. Laboratory observations of ovipositing females indicate that eggs are dispersed individually in the environment. Therefore, groups of larvae would not be expected in any natural setting. Adult emergence traps set on the floor of forests where large numbers of adult flies occur have confirmed this laboratory observation. Except for desert species, in which larval habitats are restricted to the humid lower portions of animal burrows, very few adults occur per square meter of soil. Tremendous collecting efforts involving literally tons of soil have generally yielded few larvae or pupae. Methods for extracting immature stages from soil samples include sugar floatation and filtration (Hanson 1961, Rutledge and Mosser 1972, Alencar et al. 2011). Some workers prefer using a modified Berlese funnel apparatus (Killick-Kendrick 1987, Alencar et al. 2011).

<u>Adults</u>

There are many techniques for sampling adult sand flies (Alexander 2000, Burkett et al. 2007, Junilla et al. 2011, Muller et al. 2011). In order to optimize trapping efficiency, sampling should be conducted at a standardized time and at the same locations. The number of sand flies caught strongly depends on the location and placement of the trap. Locations must be accurately identified so the trap will be placed in the same location for each subsequent survey.

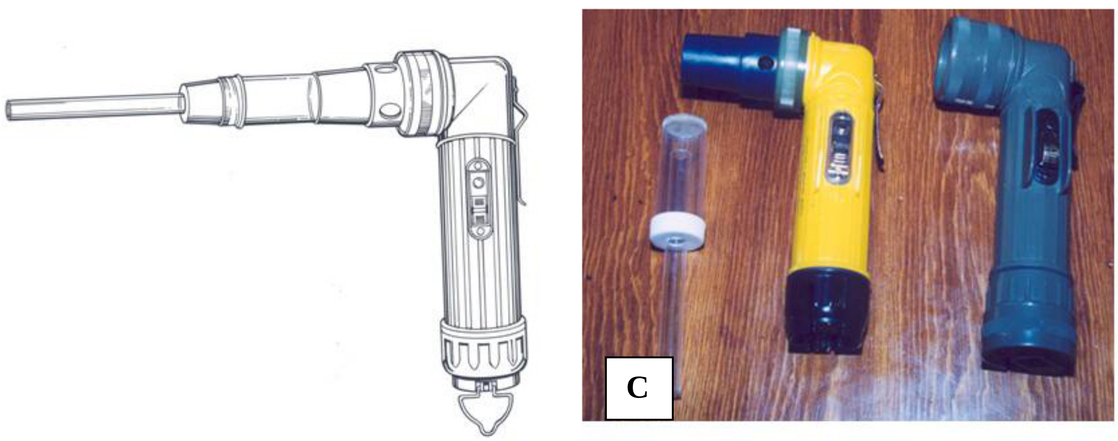
Adult sand flies can be collected from their resting sites during the early morning hours, or during their dusk-to-dawn activity cycles. Because of their small size and secretive habits, a variety of specialized methods, including direct searches, human- or animal-baited collections, and trapping are required.

Direct searches of resting sites, such as inside houses, animal shelters, animal burrows, tree trunks/buttresses/holes, caves, rock crevices and cracks in walls and wells, are especially useful for collecting live blood-fed or gravid females. The simplest way to collect resting sand flies is with an aspirator.

Aspiration (oral/mechanical) (Fig. 4-1, A-E)

Aspirators are used to collect flying insects that are too small to grab with forceps or too excitable to collect by hand. Aspirators come in several sizes and styles from multiple commercial sources, and they can be either mechanical or mouth-operated (designed for either blowing or inhalation) (Fig. 4-1 A-E). Mouth aspirators equipped with HEPA or other in-line filters are useful for removing sand flies from trap nets, or resting collections, or when consistently aspirating, and reduce the inhalation of insect particles and other particulate matter. When using a mechanical or mouth aspirator, a flashlight is essential for illuminating dark areas, and the light also may disturb the flies so that they are more easily spotted and captured. Collectors commonly disturb resting flies by moving a small branch or stick over suspected resting surfaces or by poking sticks or throwing small objects into holes to achieve the same result. Smaller mechanical battery powered aspirators can be used, but these aspirators rarely have sufficient suction power for collections. Several larger backpack or hand-held type aspirators are very useful for certain types of sampling. Commercial versions of both gas-powered and 12 V battery-powered backpack aspirators are available. Backpack aspirators use either modified leaf blowers or powerful 12 V motors attached to a 4-inch hose ending in a collection cup. Excellent hand-held larger units are also available. These latter, more powerful aspirators are excellent for making representative resting collections in a variety of habitats (e.g., edges of vegetation, rock walls, barns, inside homes, tree holes, under bridges). The gas-powered versions are better suited for outdoor use because they are noisy and tend to produce and pick up more dust. Aspirator collections are ideal for capturing large numbers of sand flies. Before deployment, check the mesh screening used on the collection vials that come with mechanical aspirators; most of these devices are designed for collecting mosquitoes and the mesh size will permit sand flies to pass through the suction fan and back into the environment (which will of course contribute to a false indication of sand fly population density). When intending to collect sand flies, replace inappropriate coarse mesh with very fine tulle mesh or 32 x 32 Lumite® screening available from suppliers such as BioQuip®. Cement the new mesh in place carefully and securely so that no gaps exist at the edges. Aspirators also can be used in conjunction with a Damasceno trap, which is a tent-like structure resembling a circular shower curtain made of bed sheeting. The trap is suspended from the end of a pole over an animal burrow, or over the space enclosed by the buttresses at the base of a large Neotropical tree. Flies that are flushed from hiding when the burrow entrance or the leaf litter between the buttresses is disturbed land on the fabric sides of the trap and can be collected with an aspirator.

Fig. 4-1. Mechanical and oral aspirators.
- **A.** Backpack mechanical aspirator (John W. Hock Co.)
- **B.** Hand-held mechanical aspirator (BioQuip).



C. Flashlight style mechanical aspirator compared with a military issue flashlight (right).

D. Traditional, mouth-operated aspirator. Photo: D. E. Bowles

E. Oral aspirator with HEPA filter (NSN 3740-01-454-2256). Photo: John W. Hock Company.

b. Indirect collecting methods

Battery-powered light traps

Different studies have shown conflicting efficacy data for the comparative effectiveness of the two main types of light traps, CDC/SSAM and BG. The addition of other attracting agents, whether UV light instead of incandescent light, carbon dioxide (CO_2), or other lures can dramatically increase trap catches, but may also affect the proportion of non-target insects in the traps, which can substantially extend processing time for separating and identifying target specimens.

CDC light trap (NSN 3740-00-134-9229) (Fig. 4-2)

The CDC light trap was developed by the U.S. Centers for Disease Control and Prevention to provide a reliable and portable sampling device. These traps can be effective for the collection of sand flies – they are small, lightweight, battery operated, and generally run on 6 volts supplied by 4 D-cell batteries or rechargeable 6-volt, gel-cell batteries (Fig. 4-3). A photoelectric switch allows for the trap to begin operating at dusk. A rain shield can be fitted to the trap for use in damp conditions. The photoelectric switch turns off the light at dawn, but the fan remains running until the battery is disconnected in order to prevent live sand flies from escaping. For field use, at least two batteries are needed for each trap; one battery can be charged while the other one is in use. CO_2 for enhancing trap effectiveness (Kasili et al. 2009, Kline et al. 2011a, b) can be supplied by a regulated compressed gas container, or through placement of dry ice in a padded envelope or insulated container that is suspended above the trap. CO_2 can also be supplied from live animals, but this may not be practical during contingency operations. In order to retain sand flies, CDC traps must be fitted with a fine-mesh net collection cage (NSN 3740-01-527-5623) if live specimens are required, but a plastic killing jar can be used if dead specimens are acceptable. In areas with heavy and persistent windblown dust, the fabric mesh of the collection cage will get clogged quickly and the microscopic shearing action of particulates on the mesh fibers will cause fraying and the development of holes that must constantly be inspected and repaired. Extreme hot-arid environments will also lead to separation of the screening cemented to the bottom of the collection cup, creating gaps for quick escape of sand flies. Routine inspection and repair may be required.

Ideally, several CDC light traps should be used in an area to conduct sand fly surveillance. Traps should be hung with the light and intake assembly at a height of 1-2 yards above the ground. Traps should not be set in areas with competing light sources, including bright moonlight (Gaglio et al. 2014). Coordinate with local unit leaders to ensure that the light produced by the traps will not affect operational security.

CDC traps can be run with or without light, but for sand flies, traps fitted with ultraviolet (UV) light are slightly more effective compared to incandescent light, and no light is inferior to any light source (Obenauer et al. 2012). In addition, black-colored traps (lid and catch-bag) baited with CO_2 catch more sand flies than traps without CO_2 or other trap configurations. Furthermore, inverting traps so that the fan blows upwards (updraft) has been shown to greatly increase the catch of sand flies (Mutero et al. 1991). Research has also shown propane combustion traps to be suitable substitutes for CO_2-baited light traps, and they offer the advantage of remaining operational around the clock for 20 days on a single tank of propane. Solid-State Army Miniature (SSAM) traps (NSN 3740-01-106-0091) (Fig. 4-4) function similarly to CDC traps and can also be used to collect sand flies. The same considerations used to ensure good collections with a CDC trap also apply to the SSAM trap.

The Collection Bottle Rotator (CBR) trap is a CDC light trap fitted with a rotating platform that holds an array of eight trap reservoirs (Fig. 4-5). A programmable timer controls a motor that rotates the platform to move a new trap reservoir in line with the suction fan at desired intervals. With the CBR trap, a fine level analysis of sand fly populations may be undertaken to determine times of peak activity. This information may be used to guide temporally precise applications of aerosol pesticides during peak sand fly population activity, and may also be used to inform local personnel when to stay away from sand fly habitat or increase vigilance with personal protective measures.

Check all battery powered traps for proper fan operation; i.e., that the fan is blowing air in the expected direction. Older traps that have been repaired and even brand new traps have been discovered with reversed wiring. When collection bags are to be removed from the trap body, make sure the fan is still running and firmly tap the side of the fan housing to force any living sand flies to the bottom of the bag before the top of the bag is disconnected from the fan housing and rapidly sealed shut. Sand flies will suddenly and rapidly escape from collection bags at the first opportunity and possibly create a hazard for the operator.

Fig. 4-2. CDC miniature light trap with collecting bag. Photo: SSG Walker, USAPHC.

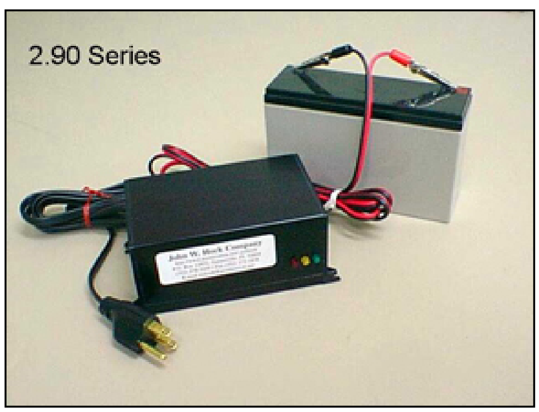


Fig. 4-3. Gel-cell 6V battery with charger.

Fig. 4-4. Solid State Army Miniature (SSAM) trap.

Fig. 4-5. Collection Bottle Rotator (CBR) trap. Photo: John W. Hock Company.

Ultraviolet (UV) tray traps (Fig. 4-6)

UV tray traps have been shown to be highly effective in capturing sand flies (Müller et al. 2011). These traps, easily constructed from aluminum, enamel or plastic trays, are topped with a mesh screen or hardware cloth to support the light and exclude large insects such as moths. A battery powered UV light is placed on top of the wire mesh. The light should be activated at dusk, and fresh batteries will allow the trap to remain on until dawn. To capture sand flies, the tray surface can be coated with mineral/castor oil, or the tray can be partially filled with ethyl alcohol or other suitable preservative. These traps are inexpensive, easy to transport, and simple to set up in the field.

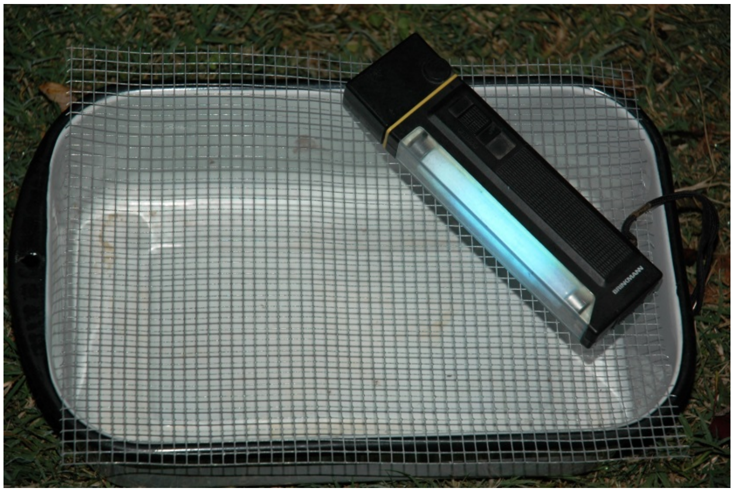


Fig. 4-6. UV tray trap. Photo: David Bowles.

BG Sentinel Trap® (NSN 3740-01-628-9326, -9324, -9327) (Fig. 4-7)

The BioGents Sentinel® trap (Fig. 4-7) can be an excellent surveillance tool for sand flies (Hoel et al. 2010). This trap mimics convection currents created by a human body, and it releases artificial skin emanations over a large surface area. The BG Sentinel trap can be used in combination with the BG-Lure (NSN 3740-01-628-9325), a dispenser that releases a mixture of non-toxic substances that are also found on human skin (ammonia, lactic acid, and caproic acid), thus making it especially attractive to hematophagous insects (Bernier et al. 2010). The addition of a CO_2 source to this trap increases its overall efficacy. A ruggedized version of this trap has been developed by the manufacturer and is currently being field-tested.

Fig. 4-7. BG sentinel trap. Photo: BioQuip®.

Intercept traps

These traps work by intercepting sand flies in flight. Any of them may be enhanced with a disposable light stick (e.g., Cyalume) placed nearby.

(1) The most common intercept traps for sand flies are oil-covered plastic sheets, which can be used in several ways. Once coated lightly with mineral oil, plastic sheets can be formed into rolls, with the oil on the inside, and stapled or clipped. The rolls can then be inserted into animal borrows to capture sand flies as they emerge from the burrows. Similarly, the oil-coated plastic sheets can be fastened to boards or other suitable solid surfaces at ground level to intercept sand flies.

(2) Oil-soaked paper cards (3x5 inch index cards) are useful for detecting sand flies. Such cards have been shown to collect adults of many sand fly species. Additionally, they are inexpensive and easily obtained. The cards can be placed at ground level near animal burrows, near tree holes, or fastened to solid surfaces to intercept flying sand flies.

(3) Sticky bottle traps are another version of an intercept trap (Wheeler et al. 1996). The performance of intercept traps is greatly improved when they are back-lighted, especially with UV light. Sticky bottle traps function no better than plastic sheets, and they require additional effort to construct.

(4) Rodent sticky traps (glue boards) may also be used if nothing else is available. This option is not the best because it reduces preventive medicine supplies for rodent surveillance and control.

Burrow traps and funnel traps

Burrow traps (Fig. 4-8) consist of a cylindrical tube with an inverted cone at one or both ends (Fig. 25). The trap is inserted in an animal burrow, tree hole, or similar location, and as sand flies emerge for feeding or other activities, they are captured in the trap. Burrow traps can be purchased from commercial sources or easily fabricated on site from plastic bottles and funnels.

Fig. 4-8. Burrow trap. Photo: Will Goldsmith, USAF.

Funnel traps are similar to burrow traps, but the widest part of the funnel faces outward and the constricted portion empties into a collection bottle (Fig. 4-9). These traps are highly useful for capturing sand flies emerging from tree holes (Comer and Corn 1991).

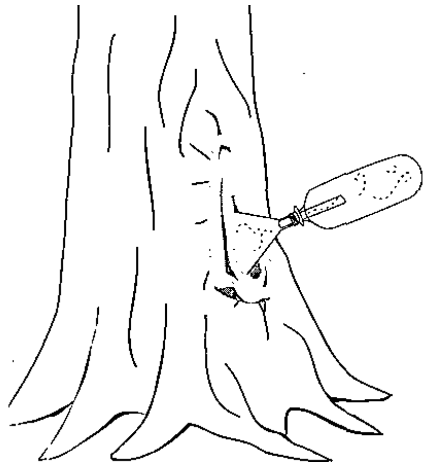


Fig. 4-9. Funnel trap placed on a tree hole.

Disney style traps (Fig. 4-10)

These traps use an animal as bait and consist of a metal tray with a cage placed in the middle to hold the chosen bait. Oil (castor or mineral) is sprayed onto the metal tray to capture sand flies attracted to the animal bait. Disney traps may not be practical for contingency purposes, but they do have merit in more controlled environments (Dorval et al. 2007).

Fig. 4-10. Modified Disney trap shown without a cover.

Soil emergence traps (Fig. 4-11)

Soil emergence traps are an indirect method used for the identification of natural breeding sites of sand flies. They are placed directly onto the soil surface of the area being sampled. Available trap designs vary in features such as size, shape, material and technique of trapping the emerging sand flies. They can be purchased from commercial sources or manufactured locally from available materials. Designs typically include a collecting bottle or sticky paper to trap sand flies that emerge into the trap. If left unaccompanied, the trap should be securely anchored to prevent it from being dislodged by wind or animals.

Fig. 4-11. A soil emergence trap available through a commercial source. Photo: bugdorm.megaview.com.tw.

Recording surveillance data

A permanent record should be kept of all sand fly surveillance data and should include the number of sand flies counted or trapped, the species observed, the collection method used, the habitat type where the collection was made, ambient environmental conditions at the time of trapping, the collector’s name, and the date. The collection method should include the time interval trapping takes place (e.g., 1800 h until 0800 h the next morning), and the date of collection should consistently indicate either the night the trap was set or the morning the trap was brought in. A standardized collection data form is recommended for this purpose. Records of sand flies trapped and counted are useful for documenting population trends. The surveyor should enter data in a spreadsheet program and prepare graphs to illustrate changes in population levels relative to the dates of pesticide applications. WRBU staff can custom design collection data forms and management tools, facilitate upload of all sand fly data to VectorMap, and provide training on request. For assistance, contact VectorMap program manager Dr. Douglas Burkett (BurkettDA@si.edu).

4-7. Handling and processing field-collected sand flies

Preservation techniques for storage and transportation of sand flies depend on the purpose for which the specimens were collected. If possible, it is best to transport adult specimens to the laboratory for identification alive in collection nets or containers placed into a cooler box lined with moist paper towels and ice packs. Typically specimens collected in traps and by aspiration will contain many other insect genera and will need to be sorted using a stereomicroscope. Trap catches should be knocked down chemically with CO_2, ethyl acetate or chloroform, or by placing collection nets or containers into the freezer (-20^{o}C) for a few minutes. Sand flies should be sorted quickly in order to preserve the DNA/RNA and stored in secure vials or tubes bearing permanent labels identifying the original collection numbers, site, date and name of collectors.

No more than ten flies should be stored in any one vial.

Storing specimens for taxonomic studies

Specimens for taxonomic studies can be preserved dry in layers of tissue paper prior to being cleared in lactophenol for identification and subsequent slide mounting in Canada balsam, Berlese, Hoyers or Euparal® fluid media (Moore & Gage 1996, Alexander 2000, Marquardt et al. 2005, Volf & Volfova, 2011). In preparing the slides, careful clearing, dissection, and arrangement of each specimen is essential to enable identification to species. Particular care must be taken to avoid folding the wings, losing antennae, collapsing the spermatheca, or improperly rotating the head, all of which can prevent identification.

Specimens can also be preserved in 70% ethanol. While this can buffer the specimens, keeping them intact, alcohol storage hardens the muscles of the insects and obscures internal structures, such as the female spermathecae, used in identification. Uncleared specimens should not be kept in alcohol for more than 6 months.

Storing specimens for DNA analysis

The optimal method for preservation of sand fly samples destined for DNA and protein analysis is in liquid nitrogen; however, most studies involving DNA-based protocols can use dried, fresh, frozen or alcohol-preserved specimens. For preservation of DNA, 95-100% ethyl alcohol can be used very effectively without immediate need for refrigeration. At room temperature, DNA specimens in alcohol remain in relatively good condition for 1 or 2 months, although they should be transferred to -70^{o}C for optimal long-term storage.

Storing specimens for pathogen/virus studies

For virus isolation or incrimination studies, sand fly adults must be kept alive, or as cold as possible, and be transported to the laboratory on dry ice or in liquid nitrogen in order to preserve viral RNA. If adults are transported alive to the laboratory, they should be placed in a freezer (-80^{o}C) and killed immediately. Samples collected using sticky traps should not be used for virus isolation studies because the oil on the surface of traps interferes with cell culture (Moore & Gage, 1996). Pathogen detection can be carried out immediately by dissection or using PCR techniques (see Section 5). For DNA detection only, commercially available preservation solutions that inactivate nucleases (such as RNALater®) can be employed.

4-8. Specimen handling and shipping procedures

Recipients of live material in the US must have a US Public Health Service entry permit and the shipper must comply with all local government and shipping company regulations.

Shipping specimens in alcohol: Specimens shipped in vials or jars should be filled to the top with 70% ethanol (for morphology) or 95% ethanol (for PCR applications) so all the air is removed, as described above. When done properly there should be no air bubbles inside the container. Each jar or vial should be wrapped to prevent contact of the containers. Cotton, tissues or cellucotton are excellent materials to pack around the containers since they also absorb alcohol should any of the containers break. Foam rubber or tissue should be placed over the vials and the boxes taped shut. If more than one specimen box is to be included in a shipping container, they should be taped securely together, wrapped in brown paper (or equivalent) and placed in the center of an appropriately sized shipping container. The outer container must be large enough to allow for 3-4 inches of packing material on all sides (including top and bottom) of the specimen boxes. Styrofoam peanuts and wood excelsior are the most common packing materials used. However, if these are not available, shredded paper or crumpled newspaper may be used. The object is to absorb shock and prevent the specimen boxes from shifting and coming in contact with the outer shipping container. If the package is to be mailed internationally, it should have the necessary customs declaration form and the contents should be identified as "DEAD INSECTS FOR SCIENTIFIC STUDY - NO COMMERCIAL VALUE."

Shipping dry adults: Dry adults should be placed in waxed pill boxes using fine, lightweight soft tissue or lens paper for cushioning. The paper should be cut slightly larger than the box to prevent settling and compacting of the specimens. The specimens should not touch each other and can be loosely packed between single sheets of paper. The pill boxes should be placed in a larger box along with packing material to fill any excess space, and then packed as described above.

Shipping insects on dry ice/dry shipper: Samples shipped on dry ice and/or in dry shippers (liquid nitrogen) are potentially biohazardous, and shipment can be tricky. Recipients in the USA must have valid USDA/CDC permits and shipments must be made to the exact name and address listed on the license. Complete all required paperwork prior to preparing specimens, and establish clear communication with the appropriate shipping office (e.g., FedEx) in advance to ensure full understanding of the nature of the frozen shipment. When sending cold-chain samples, ensure that specimens are packed with a generous supply of dry ice in a sealed polystyrene container, allowing for unexpected transit delays. Dry shippers can be transported on aircraft as part of luggage allowances, but be sure to obtain written permission from the airline before arrival – and always have a contingency option for storage of samples in case of problems.

SECTION 5. SAND FLY DISSECTION AND DETECTION OF SAND FLY-BORNE PATHOGENS

Various methods can be used to detect pathogens in sand flies, including dissections and multi-locus electrophoresis (for *Leishmania* spp.), molecular detection including conventional PCR, reverse transcriptase PCR (RT-PCR), real-time PCR (for *Leishmania* spp., phleboviruses and bacteria), and in vitro cultivation and cell culture inoculation (*Leishmania* spp., phleboviruses and bacteria). In vitro cultivation and cell culture inoculation are extremely time-consuming for sand flies because the natural infection rate is very low for *Leishmania* spp. and phleboviruses, ranging from 0.02% to 0.68% (Zhioua et al. 2010, Ergunay et al. 2012, 2014). Pooling is frequently used to decrease costs and allow processing of a higher number of specimens. However, Alten et al. (in press) warn that pooling may also decrease overall detection sensitivity at such low incidence.

5-1. Detection of *Leishmania* spp. in sand flies

The incrimination of a sand fly species as vectors of *Leishmania* spp. is based on a series of widely accepted criteria: (a) the vector must feed on humans; (b) in zoonotic leishmaniasis, the vector must also bite the reservoir host(s); (c) the vector must be infected in nature with the same *Leishmania* species that occurs in humans, and this must be ascertained by comparison of isolates using isoenzymes or DNA; (d) the vector must support the complete development of the parasite after the infecting blood meal has been digested; and (e) the vector must be able to transmit the parasite by bite to a susceptible host while taking a blood meal (Killick-Kendrick 1990). Given the inherent difficulties in detecting wild-caught sand flies with salivary gland infections and finding susceptible host animals to subject to infection, Maroli et al. (2012) suggest that the minimal requirements for robust vectorial incrimination should instead be: (a) overlapping geographical distributions of the vector and the human disease; (b) evidence that the vector feeds on humans; and (c) evidence that the vector supports natural gut infections with promastigotes of the same *Leishmania* species that occurs in humans.

Sand fly dissection protocols

The detection of *Leishmania* promastigotes in sand flies is normally carried out by dissection and examination under a stereomicroscope. Sand flies should be fresh and large numbers (e.g., 25-50) are needed, as natural infection rates are low.

Sand fly dissection techniques for the salivary gland and gut dissections are listed in detail below (Lawyer et al. 2011). In advance, prepare dissecting pins by inserting size 000 insect pins into the end of a wooden applicator stick. Soften the end of the stick by soaking in water for an hour, then, using a jeweler's forceps, drive the blunt end of the pin into the softened end of the applicator stick. Prior to starting dissections, ensure access to the following:

- Dissecting pins
- Sterile microscope slides and cover glasses (18 x 18 mm)
- Soap solution in 15 ml cone-bottom tube for immobilizing sand flies
- Beaker and piece of fine-meshed screen
- Phosphate buffered saline (1xPBS) or sterile saline for rinsing
- 1cc syringe of PBS
- Dissecting microscope
- Compound microscope

Salivary gland dissections for *Leishmania* vector implication studies

Transfer the flies into the tube of soap solution and shake gently. Pour the contents of the tube onto a fine-mesh screen stretched over the mouth of a large beaker and rinse with tap water until the soap is gone. Using a dissecting pin, lift the flies from the mesh screen and place them in a small Petri dish containing 1xPBS to rinse. With the 1cc syringe, apply a drop of PBS to the glass microscope slide. Place a freshly rinsed sand fly in the drop of PBS on the microscope slide. With two dissecting needles (bent one in the dominant hand and straight one in the other hand), remove the sand fly's legs. Steady the fly by piercing the thorax at the base of the wing with the straight pin and holding it against the glass. With the bent pin in the dominant hand, remove the sand fly's head. When the head is pulled from the body, the salivary glands should come with it and will be visible at the back of the head. Tease away the glands with the dissecting pins (Fig. 5-1). Aspirate the glands into a 1cc syringe with PBS and transfer to a small, labeled Eppendorf tube for storage.

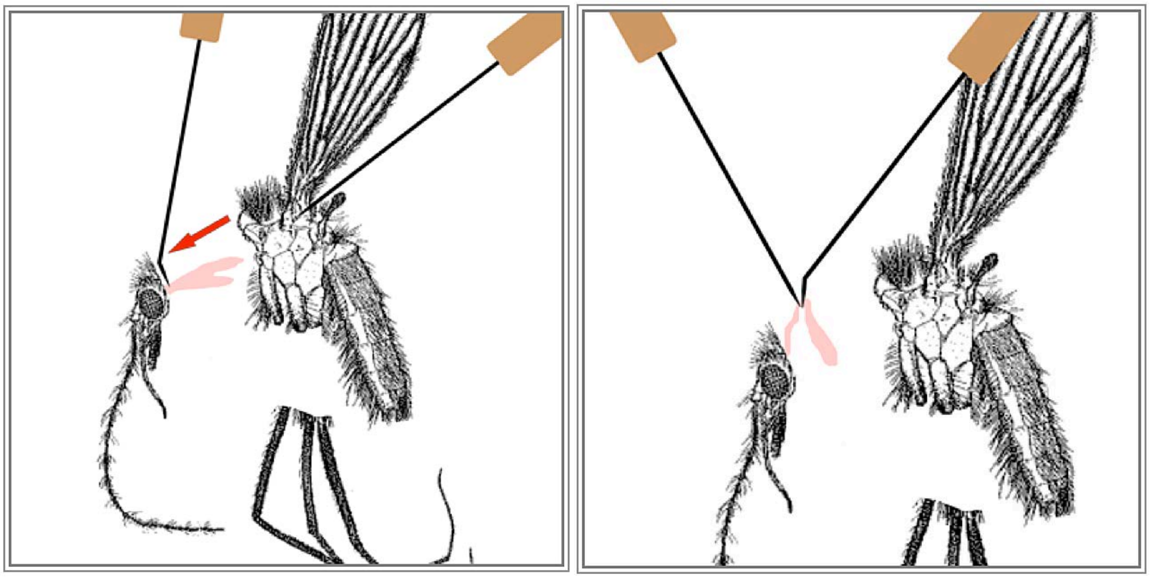


Fig 5-1. Dissection of sand fly salivary glands. Illustration J. Stoffer (WRBU), after El-Hossary (2006).

Sand fly gut dissections for the detection of *Leishmania* promastigotes

Transfer flies to the tube of soap solution and shake gently. Pour contents of tube onto a fine-mesh screen stretched over the mouth of a large beaker and rinse with tap water until soap is gone. Using a dissecting pin, lift the flies from the mesh screen and place them in a small Petri dish containing 1xPBS to rinse. With the 1cc syringe, apply a drop of PBS to the glass microscope slide. Place a rinsed sand fly in the drop of PBS on the microscope slide. With two dissecting needles (bent one in the dominant hand and straight one in the other hand), remove the sand fly's legs (Fig. 5-2, left). Steady the fly by piercing the thorax at the base of the wing with the straight pin and holding it against the glass. With the bent pin in the dominant hand, remove the sand fly's head (Fig. 5-2, center). Keeping the pin in the left hand in position to secure the fly, press the tip of the bent pin horizontally against the tip of the abdomen between segments 7 and 8 and pull firmly but slowly and steadily downward (toward you) until the gut comes out of the abdomen (Fig. 5-2, right). If the end of the gut breaks off, place the bent segment of the pin horizontally across the anterior abdomen and squeeze the gut out of the abdomen like you would toothpaste out of a tube. Often the diverticulum (crop) breaks off and you must "fish" it out of the anterior abdomen. Place a cover glass over the gut and examine the gut from one end to the other for parasites under a compound microscope (Lawyer et al. 2011). The specific identity of the pathogen can be verified using molecular techniques (see Section 5-2).

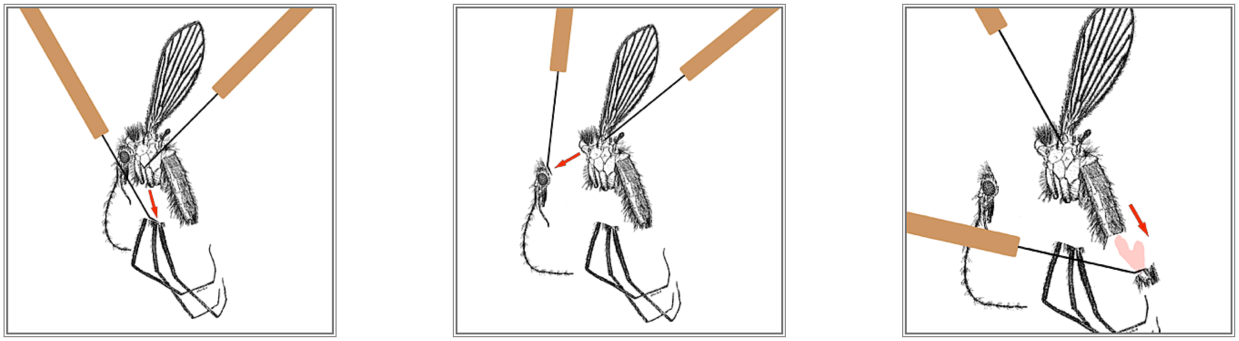


Fig. 5-2. Sand fly gut dissections for the detection of *Leishmania* promastigotes. Illustration: J. Stoffer (WRBU), after El-Hossary (2006).

5-2. Detection of *Leishmania* spp.

Because of their high sensitivity and specificity, molecular techniques, including PCR, PCR-restriction fragment length polymorphism (PCR-RFLP) and probe hybridization have been successfully used to detect, identify and characterize *Leishmania* species in humans and other vertebrate reservoir hosts (Volpini et al. 2004, Carvalho et al. 2008, Saraiva et al. 2010). Key DNA targets for parasite differentiation include the rRNA gene, minexon-derived RNA gene, repeated genome sequences and the kDNA minicircle (Aransay et al. 2000, Paiva et al. 2006). A single parasite is sufficient for detection by PCR (Kato et al. 2007).

To facilitate vector incrimination studies in New World sandflies, Kato et al. (2007) published a relatively cheap yet effective method for screening large numbers of sand flies for *Leishmania* infection, based on PCR amplification of a highly specific target region (c. 600bp of the *Leishmania* minicircle kinetoplast) using the L.MC-1S and L.MC-1R primers designed in Kato et al. (2005). The mitochondrial *cytochrome b* gene has proven useful for the reliable differentiation of *Leishmania* spp., thus *Cyt B* fragments were amplified and sequenced from those sand flies found to be positive for the *Leishmania* minicircle DNA. Resultant *Cyt B* sequences were blasted against many available *Leishmania* parasite DNA sequences in GenBank to determine the parasite species present. A PCR-RFLP assay based on previously determined sand fly species' diagnostic 18S rRNA sequences, digested with the restriction enzymes *Afa*I or *Hin*f1, was used to identify Neotropical vectors (Kato et al. 2007).

Antigen assay dipstick for detection of *Leishmania major*

An antigen assay "dipstick" (VectorTest® *Leishmania major* Antigen Assay, Cat. No. LMAJ-K020) is available from VectorTest® Systems Inc. for the rapid detection of *L. major* in sand flies.

5-3. Detection of sand fly fever viruses

Viral RNA detection via PCR and inoculation of cell cultures are the two most widely used techniques for virus detection in sand flies. Viruses can be cultured from preserved sand flies. The flies destined for cell culture should be killed by freezing and stored at -80^{o}C without a break in the cold chain. They must be shipped on dry ice to a BSL-2 or higher facility with culture capability. If cultured viruses are requested, the results will not be available in less than 1 week and generally require more than a month. Unlike the other assays, culture methods can detect a wider range of pathogens but are not regularly used for threat assessments because of the cost and increased biosecurity.

Many molecular methods have been employed to detect viruses in sand flies, including standard PCR, single or multiplex real-time PCR (Sanchez-Seco et al. 2003). Sand fly fever viruses include several members of the *Phlebovirus* genus (family Bunyaviridae), Chandipura (genus *Vesiculovirus*, family Rhabdoviridae) and Changuinola (genus *Orbivirus*, family Reoviridae) (Depaquit et al., 2010, Plyusnin et al. 2012), and molecular tools target either genera-specific or species-specific target regions (Marquardt et al. 2005). Bichaud et al. (2014) reported discovery of 23 phleboviruses in sand fly populations across Europe, six of which were new to science, through the combination of cell culture and next generation sequencing. There is some evidence that viral DNA can be detected by PCR in dry sand flies up to a week after collection; however, cold-chain is still the preferred method if possible.

Antigen assay dipsticks for sand fly fever viruses

Toscana virus and Naples sand fly fever viruses can be detected in sand fly vectors using an antigen dipstick assay (SFFV Antigen Assay from VectorTest Systems Inc., Cat. No. SFFV-K020), and results can be read within 15 minutes. Initial tests conducted by Dr. Will Reeves (USAF) show that the assay sensitivity varies and can give false negative results. There are no other dipstick assays for viruses in sand flies. Flies should be killed by freezing or with chemicals, but flies must be stored dry or frozen prior to testing.

5-4. Detection of *Bartonella bacilliformis* in sand flies

Bartonella bacilliformis, the causative agent of bartonellosis, can be cultured from preserved sand flies (see Section 5-3), but PCR techniques appear highly sensitive and will most likely form the basis of routine epidemiological screening. Sand flies collected specifically for *Bartonella bacilliformis* screening can be stored in 75%-100% ethanol. They should be shipped and tested within a few weeks of collection. Wild-caught flies collected in a *Bartonella* disease focus in Peru were incriminated by a highly specific Real-Time PCR assay, capable of detecting <100fg of *Bartonella* DNA (Romero 2004). Of the 472 pools of five *Lutzomyia verrucarum* sand flies tested, 13 (2.75%) were found to be positive for *Bartonella* (Romero 2004). Based on surveying known samples, the sensitivity of the test was 100% and specificity was shown to be 91.11%.

SECTION 6. SAND FLY CONTROL

6-1. Introduction to Control of Phlebotomine Sand Flies

Combinations of control methods are essential in sand fly suppression programs but, even when applying Integrated Vector Management (IVM; www.who.int/neglected_diseases/vector_ecology/en/) or Integrated Pest Management (IPM) techniques, it is difficult to achieve satisfactory control of sand fly populations (Alexander and Maroli, 2003, Coleman et al. 2011, Warburg and Faiman 2011). Area-wide habitat modification can be effective in disrupting host ecosystems and reducing the density of sand flies, for example, by ploughing or flooding terrain harboring the immature stages, but such approaches are ecologically damaging and the breeding sites are seldom known. Usually the larval habitats are in animal burrows, soil fissures, termite nests, caves, or the organic litter in animal shelters. Pesticide application alone is not sustainable, being limited by time and resources, and the uncertainty of target sites for effective treatment. Without thorough understanding of where to apply source reduction, adulticiding with space sprays and residual pesticides is the most effective immediate method for reducing adult sand fly populations and must be considered when a disease threat exists (Kitchen et al. 2009). Moreover, sand fly problems may originate in areas away from a camp and outside the control of military personnel. This limits the options for control techniques. Population reduction by adulticiding will never eliminate sand fly populations or the risk of disease transmission. Personnel must simultaneously employ personal protective measures to further reduce risk.

Pesticide application must always be done according to U.S. Environmental Protection Agency (US EPA) label guidelines. Pesticide labels are legal documents. Failure to follow label instructions is a violation of federal law. Also, a memorandum from the Joint Chiefs of Staff dated 1 February 1999 mandates that, except in an emergency as determined by the Joint Task Force Surgeon, only pesticides on the DoD Approved Pesticides List can be used by U.S. military personnel in CONUS and on deployment. All pesticides noted in this manual are US EPA approved and authorized for use by DoD-certified pesticide applicators.

Several methods of pesticide application can be used simultaneously in sand fly control (e.g., baits or residuals and space sprays). The choice of application method is dependent upon a range of factors. In most cases, pesticides and pesticide baits should only be used outdoors. In deployed situations, the type and amount of application equipment are often deciding factors. Good planning is necessary to ensure pesticide formulations match application equipment, and that the planned method of application is adequate to accomplish the task. Ordering information for pesticides and pesticide dispersal equipment is available in Armed Forces Pest Management Board Technical Guide 24 (TG24): Contingency Pest Management Guide, available at the AFPMB web site www.afpmb.org/content/technical-guides.

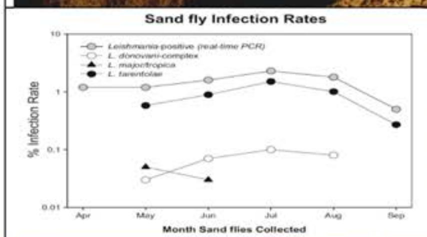
CASE STUDY: Impact of Sand Flies and Leishmaniasis on U.S. Military Operations.

Significant Impact on Military Operations:
Tallil Airbase: Operation Iraqi Freedom (OIF), 2003-2010

dry scrubland, with abundant sand flies and rodent hosts, cleared for DoD tent cities: numbers of sand flies remained high, and it was difficult to see where they came from

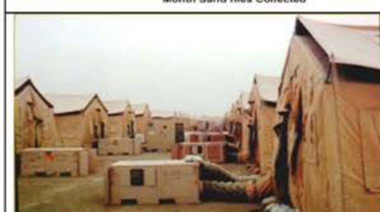
During the first year of OIF, many of the 24,000 military personnel stationed in or transitioning through Tallil Airbase in southern Iraq experienced 100s to 1000s of sand fly bites/night, with 2% *Leishmania* infection rate. Both cutaneous and visceral leishmaniases (CL & VL) are endemic to Tallil area and the local Ministry of Health office in nearby Nasiriyah reported an on-going outbreak of the more dangerous VL. With ambient 100-130° temperatures our Forces worked and slept in tents without A/C. Vector control efforts with residual and ULV sprays, rodent control and habitat destruction, all failed to quickly reduce sand fly populations. The over-extended logistical system could not deliver adequate numbers of bednets or repellents for personal protection against sand fly bites. This raised concerns in the military medical community over risks of an unprecedented wartime outbreak of leishmaniasis or sand fly fever.

24% *Phlebotomus papatasi*
30% *Phlebotomus alexandri*
45% *Sergentomyia* spp.



fore-arm exposed to sand fly bites

upper arm without bites where covered by shirtsleeve



AF Tent City

Army Tent City

Rodents and Sand Flies Breeding Under Tents?

Although U.S. military forces deployed in Iraq experienced numerous complications from sand fly bites, secondary infections and reported 823 cases of leishmaniasis (10 visceral) in 2003, the impact on combat forces and military operations could have been far worse. The Tallil AB experience taught us that unexpected vector-borne diseases are still inevitable in wartime. Therefore we need better public health pesticides and application technologies, repellents and personal protective gear, with enhanced capabilities for surveillance and identification of phlebotomine sand flies. Hence R&D efforts over the last decade have been enhanced to improve our military ability to prevent transmission of sand fly borne pathogens. Many of the control tactics and equipment presented in this TG have been improved in response to difficulties of combating phlebotomine sand flies during military operations in the Middle East.

Refs: Burkett et al., 2007; Colacicco-Mayhugh et al., 2011; Coleman et al., 2006; 2007; 2009a,b; 2011.

6-2. Insecticidal Baits

a. Attractive Toxic Sugar Baits (ATSB), a.k.a. Attractive Targeted Sugar Baits

ATSBs consisting of insecticidal fruit juices or sugar solutions are being introduced for controlling sand flies in the Middle East, and for mosquito control operations in other countries. The first ATSB product launched in the USA had garlic as the active ingredient, to be exempt from EPA regulations, and therefore was not approved for military use. In recent ATSB research studies, boric acid (1.0% w/v), spinosad (Tracer®; Dow Agrosciences, 0.04% w/v), dinotefuran or other insecticides have been added as oral toxins in solutions used at experimental sites. ATSBs can be sprayed on vegetation or on barrier fences to combat sand flies, at least in arid areas where attractive plants are scarce or absent. An ATSB bait station with boric acid has been registered with EPA but has not yet been marketed in the USA, although it is commercially available in Israel (www.wsthm.com).

b. Rodent feed-through baits: targeting sand flies via their rodent hosts

Insecticidal baits for consumption by rodents can be effective against sand fly larvae via rodent feces in fly breeding sites in rodent burrows, or when female sand flies feed on rodents having insecticide in their blood (Mascari et al. 2013). The first reported success of this method employed rodent oral bait for systemic control of *Ph. papatasi* vectoring zoonotic *L. major* in jird (gerbil-like rodent) burrows in Tunisia. After that success, which utilized 'Kaput® rodent flea control bait' containing 0.025% imidacloprid as the active ingredient (EPA Reg #72500-17), a Supplemental label was approved for sand fly control. The manufacturer is now developing a more potent version of the product.

6-3. Adulticides Recommended for Sand Fly Control

Sand flies are rarely physiologically resistant to the standard adulticides employed for mosquito control. Therefore, sand fly control is generally similar to mosquito adulticiding. Although sand fly adults often seem to evade space sprays, applications of the following insecticides have been effective for controlling sand flies in some situations:

For residual applications:
Talstar P® (bifenthrin, NSN 6840-01-525-6888)
Pestabs® (λ-cyhalothrin, NSN 6840-01-431-3357)
Demand® CS (λ-cyhalothrin, NSN 6840-01-428-6646)
Demo®n WP (cypermethrin, NSN 6840-01-390-4822)
Aqualure® 20+20 (permethrin, PBO; NSN 6840-01-606-8581)

For treatment of rodent burrows:
Delta Dust® (deltamethrin, NSN 6840-01-431-3345)

For ULV or thermal fog space spray (aerosol) applications:
Aqua-K-Othrine® = DeltaGard® 2% ULV formulation (EPA registration pending)
Fyfanon® ULV (malathion, NSN 6840-01-169-1842 [5 gal] and 6840-00-926-1481 [55 gal])

Aqualure® 20+20 (permethrin, PBO; NSN 6840-01-606-8581)
Zenivex® E20 (etofenprox; NSN 6840-01-573-4964)
Duet® (prallethrin, sumithrin, PBO; without NSN)
d-Phenothrin 2%; NSN 6840-01-412-4634

For misting applications: (see below in Section 5-5 and Fig. 5-4.)
Sector® (permethrin, PBO)
Essentria® (botanical)

6-4 Pesticide Application Equipment

Residual applications:

Two effective hand-held sprayers for applying residual pesticides on a small scale are the hand-pressurized 2 gallon hand sprayer (NSN 3740-00-641-4719), and the engine-powered backpack mist blower (NSN 3740-01-463-0147). These small sprayers are ideal for treating focal areas, such as uniforms, bed nets, tentage, camouflage netting, shade cloth, and HESCO® barriers (Fig. 6-1). The 2 gallon hand sprayer should be present in Field Sanitation Team (FST) kits (NSN 4540-01-578-4352; per Appendix C of FM 4-25.12 Unit Field Sanitation Team) and both FST and non-preventive medicine personnel may readily be trained to apply residual pesticides as needed.

For larger areas, such as perimeter vegetation or HESCO® perimeter, or even an entire installation, vehicle-mounted hydraulic sprayers are ideal. To be most effective, treatment of these surfaces should be conducted where personnel are most commonly found, or where obvious sand fly habitat exists. Two vehicle-mounted sprayers that are known to be effective for barrier treatments against mosquitoes on desert vegetation, and are expected to be effective against sand flies, are the Spectrum electrostatic sprayer (without NSN) (Fig. 6-2) and the Buffalo Turbine standard mist blower (NSN 3740- 00-901-0720). The Swingfog SN101 (without NSN) thermal fogger may be used with Aqualure 20+20 for barrier treatment on desert vegetation. Care should be taken with all thermal fog equipment to not ignite dry vegetation or flammable materials; keep shovels and fire-extinguishing materials on hand at all times.

Fig. 6-1. Treating HESCO® units with Pestabs residual λ-cyhalothrin using a Stihl SR 450 backpack sprayer at an experimental plot in the Rift Valley, Kenya. Photo: Seth Britch, USDA.

Fig. 6-2. Treating sparse desert vegetation with Talstar residual bifenthrin using a Spectrum electrostatic sprayer at an experimental plot in southern California, USA. Photo: Todd Walker, USN.

ULV or thermal fog space spray (aerosol) applications:

For small or medium-sized areas:

Terminator ULV (NSN 3740-01-525-7600) (Fig. 6-3)

For medium- to large-sized areas:

Grizzly ULV (NSN 3740-00-375-9154)

Swingfog SN 101 thermal fogger

Care should be taken with all thermal fog equipment to not ignite dry vegetation or flammable materials; keep shovels and fire-extinguishing materials on hand at all times.

6-5. Outdoor Spray Applications

Aerosol Ultra Low Volume (ULV) and thermal fog insecticide applications (space sprays) to control sand flies can be effective (Britch et al. 2011a, 2010b). Ideally, applications should be made at dusk with winds only 1-2 mph. However, challenges in the field, including operational security, logistical and personnel constraints, and local meteorology may make standard guidelines prohibitive and impractical. If opportunities arise for pesticide sprays at other times and under less than ideal conditions, these opportunities should be exploited. Traditional aerosol application times (i.e., at dusk) attempt to target sand flies in flight, but recent studies have demonstrated local population suppression when sprays are conducted as early as the mid to late afternoon (Britch et al. 2011a). Pesticide sprays may reach sand flies at rest in hidden areas in vegetation, structures, or burrows at any time of day (Fig. 6-3). Spray nozzles should be set at a downward angle of 45^{o} in order to place spray clouds in gaps and cracks in low walls and other structures. For larger structures, such as HESCO® barrier walls or perimeter vegetation, nozzles should be set at 90^{o}.

Fig. 6-3. An aerosol application of Duet® (sumithrin, prallethrin, PBO) with a truck-mounted Terminator ULV against sand flies in an experimental plot in the Rift Valley, Kenya. Photo: Seth Britch, USDA.

Thermal fog or ULV pesticide applications are the only assured means of immediate control. Space sprays are effective at killing flying insects over large areas, but results are often short-lived, as insects move in from unsprayed areas. More long-term control strategies should be implemented as soon as possible to reduce reliance on space sprays, which are expensive and labor intensive.

ULV systems require specially formulated pesticides for application through thermal and cold foggers. The theory of application is to fill the air with a cloud of small droplets. Droplets in the cloud are dense enough that a lethal dose will impinge on many or most of the target insects in flight or at rest within the treated area. Space sprays are carried through the target area by wind, but if wind speeds are too high the sprays will quickly spread out and be too dispersed to be toxic. Shortly after treatment, all pesticides either move or settle out of the treated area. Because pesticides do not remain in the treated area, fly populations may begin to rebuild immediately after treatment. If the treatment area is too small, re-invasion can be almost immediate. Given the prospects for re-invasion, frequent reapplication is often necessary unless or until more sustainable methods are implemented.

Treating rodent burrows with a thermal fogger, or application of insecticidal dust or pesticide-treated foam to the interior of small mammal burrows, can be effective for controlling sand flies.

Pesticide misting systems may be set up on camp perimeters to reduce movement of sand flies into a protected area (Fig 6-4). Misting systems are not in the DoD pest management inventory and have not been fully evaluated or approved for use by the AFPMB at this time. Refer to http://www2.epa.gov/mosquitocontrol/mosquito-misting-systems for more information.

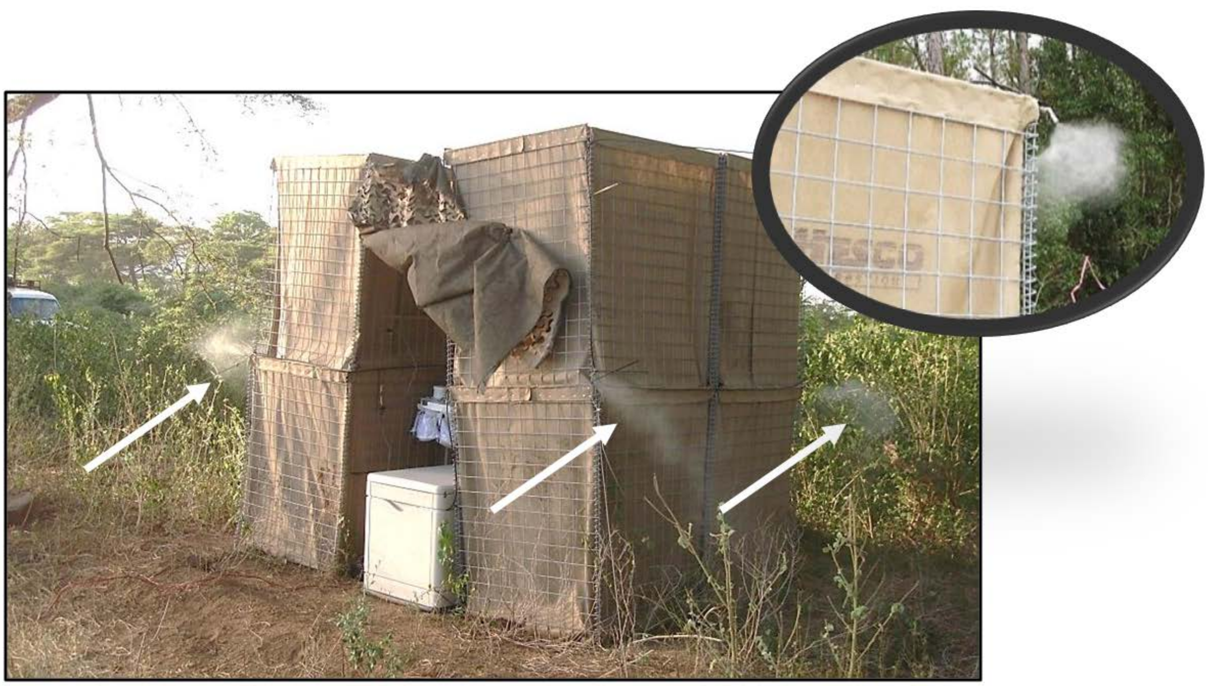


Fig. 6-4. Misting system fitted to experimental HESCO® units targeting sand fly vectors of *Leishmania* in the Rift Valley, Kenya. Arrows indicate mist plumes; inset shows detail of nozzle and spray. Photo: Seth Britch, USDA.

6-6. Indoor Space Sprays

Buildings and tenting can be disinsected by spraying d-phenothrin for 10 seconds per 1,000 ft^3 (30 m^3), per label instructions. Tents require repeated spraying if attempts are not made to exclude sand flies. The d-phenothrin aerosol can be almost 100% effective indoors when used to augment effective exclusion measures. For interior walls and entryways, a fine spray should be used. Indoor application of pesticides should only be conducted when personnel are absent, and personal items such as bedding should not be sprayed (have personnel cover bedding with a poncho or equivalent). Personnel should not return to structures until after the spray application has completely dried. Air handling equipment should be turned off prior to spraying.

6-7. Indoor Residual Spraying (IRS)

As in malaria vector control, IRS can be very effective against endophilic (indoor-resting) sand fly species. Historically, VL (kala-azar) was almost eliminated from Asia by IRS campaigns with DDT for malaria control. Recently, the resurgence of kala-azar, transmitted by *Phlebotomus argentipes* in the Indian subcontinent, has been turned back by IRS with pyrethroid insecticides.

Indoor residual sprays are applied with the 2-gallon hand can and backpack sprayers described in Section 6-4. Hand cans are well suited for small jobs where all areas to be treated are easily accessible (Fig. 6-5). Motorized backpack sprayers are necessary where pesticides must be dispersed over large areas.

Fig. 6-5. Preparing Hudson 2.5 gal can sprayers to treat bed nets with Pestabs (λ-cyhalothrin). Photo: Seth Britch, USDA.

6-8. Barrier Treatments

Similar to IRS, insecticidal barrier treatments target adult female sand flies resting on outdoor surfaces, moving toward vertebrate hosts, or digesting blood meals. Sand flies touching the residual pesticide receive a lethal or sub-lethal dose that will either interrupt host-seeking and biting behavior or eventually kill them. Many materials commonly found in the field during U.S. military operations, such as HESCO® blast walls, tent exteriors, sand bags, camouflage netting and shade cloth, are suitable for treatment with residual pesticides to form a protective barrier (Britch et al. 2010a, 2011b), and these materials may be treated and stored in the rear before shipment to operational areas (Fig. 6-6). Perimeters of desert, temperate, and tropical vegetation may also be treated to reduce movement of sand flies into a defined area (Britch et al. 2009). Sections 6-3 and 6-4 detail formulations and equipment that are effective for creating barrier treatments. The Pesticide App available for iOS and Android platforms from the Armed Forces Pest Management Board at http://www.afpmb.org/content/pesticideapp contains detailed information on the expected longevity/efficacy of residual barrier treatments on a variety of surfaces in a range of environments (Britch et al. 2014). Surfaces should be retreated in the field as needed and supplemented with aerosol space sprays (Sections 6-5 and 6-6) and consistent use of PPM and PPE (Section 6-9).

Fig. 6-6. Treating ULCANS camo netting with Demand CS residual λ-cyhalothrin using a Stihl SR 450 backpack sprayer (left), and treating desert vegetation with Talstar bifenthrin using an Electrolon electrostatic backpack sprayer (right) at experimental plots in southern California. Photos: (L) Seth Britch, USDA; (R) Wayne Wynn, USDA.

6-9. Personal Protective Measures (PPM) and Personal Protective Equipment (PPE)

Precautions for personal protection are crucial for avoiding nuisance bites and prevention of disease transmission. Specific, detailed guidance for PPM in contingency operations is not presented here but can be found in Technical Guide 36, Personal Protective Measures Against Insects and Other Arthropods of Military Significance, and Technical Guide 48, Contingency Pest and Vector Surveillance. A permethrin-treated uniform that is properly worn, in conjunction with consistent use of an appropriate skin-based repellent, can provide almost complete protection from sand fly bites.

The efficacy of using permethrin-impregnated uniforms for preventing malaria and leishmaniasis was determined in soldiers on patrol in Colombia (Soto et al. 1995). In that randomized study, soldiers were issued impregnated uniforms or uniforms washed in water. Malaria was contracted by 3% of the soldiers wearing impregnated uniforms, and 14% of those wearing untreated uniforms. Leishmaniasis was contracted by 3% of soldiers wearing impregnated uniforms, and 12% of soldiers wearing untreated uniforms. The reduction was highly significant for both diseases, indicating that the use of permethrin-impregnated uniforms provided partial protection against both malaria and leishmaniasis by reducing the soldiers' exposure to bites by malaria vector *Anopheles* spp. and *Leishmania* vector sand flies. However, in another study with the Iranian military (Asilian et al. 2003), permethrin-treated uniforms worn without simultaneous use of skin-based repellent provided no protection from transmission of *Leishmania* to soldiers in the field when compared to untreated uniforms and no skin-based repellent.

Troops in the field should resist the temptation to wear PT shorts and short-sleeved shirts while lounging outdoors during morning or evening hours before or after duty. Similarly, troops should resist the temptation to sleep without the use of a bed net, in particular if sleeping uncovered with minimal clothing. The mesh size of the standard mosquito net, especially older stocks of these, may not be sufficient to keep out sand flies (NSN 7210-00-266-9736/9740); however, the more modern pop-up style bed net (e.g., NSN 3740-01-516-4415) has a small mesh size to exclude sand flies and is also factory treated with permethrin.

6-10. Techniques for Preventing Sand Fly Entry into Tents or Hardened Structures

One of the riskiest areas for sand fly bites is within tents or hardened structures because people tend to let their guard down by not consistently using PPM or PPE (Section 6-9 above) and by not properly sealing the area from entry of sand flies. Tents include the full range of soft-sided shelters from small two-person tents to large tent complexes, and can include tents heavily modified with thick spray-on foam coverings, raised flooring, and interior plywood partitions. Hardened structures may include bunkers, conex containers, aircraft hangars, local nation buildings or ancient structures pressed into service as living or office areas, or containerized housing units (CHUs) and plywood buildings erected by contractors or military construction units (Navy Seabees, Army Engineers, RED HORSE). All these structures should be inspected for rodent habitat (for instance, beneath flooring or in spaces between sandbags) or for cracks and channels that could allow rodent movement into the structure, all of which will be exploited by sand flies to reach human hosts. An easy procedure that can be presented to troops by First Sergeants is to carry out the "light leak" test: have personnel douse all light sources within the structure during daylight hours, wait several minutes for eyes to adjust, and then begin to carefully inspect for pinholes or cracks where light is showing, in particular looking under beds, at floor and ceiling corners, around air conditioning units, and around doors and windows. Wherever light is showing, securely fill gaps with duct tape or spray foam. Sandbags around tents and bunkers should be replaced when they begin to break open, and, if possible, treat all sandbags with residual insecticide (Section 5-8) before they are filled and placed. Also if possible, add vestibules to structures so that personnel pass through two doors that are difficult to open simultaneously. Many tactical structures already have these in place to prevent light leaks at night. Biting insects tend to collect in vestibules, so particular attention should be paid to treating the interior of vestibules with residual insecticides. Sand fly activity in structures with effective air cooling systems will be low but not absent, and sand fly activity should be expected to increase rapidly if air cooling systems malfunction even for a couple of hours. At minimum, personnel should be urged to not wear shorts or short-sleeved shirts while at work or rest within structures in sand fly-*Leishmania* endemic areas.

6-11. Insecticidal Dog Collars

Under PPM guidelines in TG36, Section 2.13 warns against wearing insecticidal pet collars and ear tags on the human body. However, they are appropriate for protection of domestic animals against fleas, ticks, and vector-borne diseases. Some types of pet ‘flea collars’ provide treatment that spreads through the fur to give extensive protection of the animal’s body against ectoparasites. In countries or regions (e.g., Brazil, France, CENTCOM) where domestic dogs are important reservoirs of zoonotic leishmanias, one brand of insecticidal dog collar has been introduced for protection of dogs against sand flies and the risk of *Leishmania* infection. The deltamethrin-impregnated Scalibor® Protector Band (Fig. 6-7) is approved by the US EPA (Reg No. 68451-1) for use on dogs in the U.S. to protect against fleas, ticks, and tick-borne agents such as *Borrelia burgdorferi*, which causes Lyme disease. It is advisable for military working dogs to wear the Scalibor® Protector Band when deployed where they are likely to be bitten by sand flies carrying *Leishmania*.

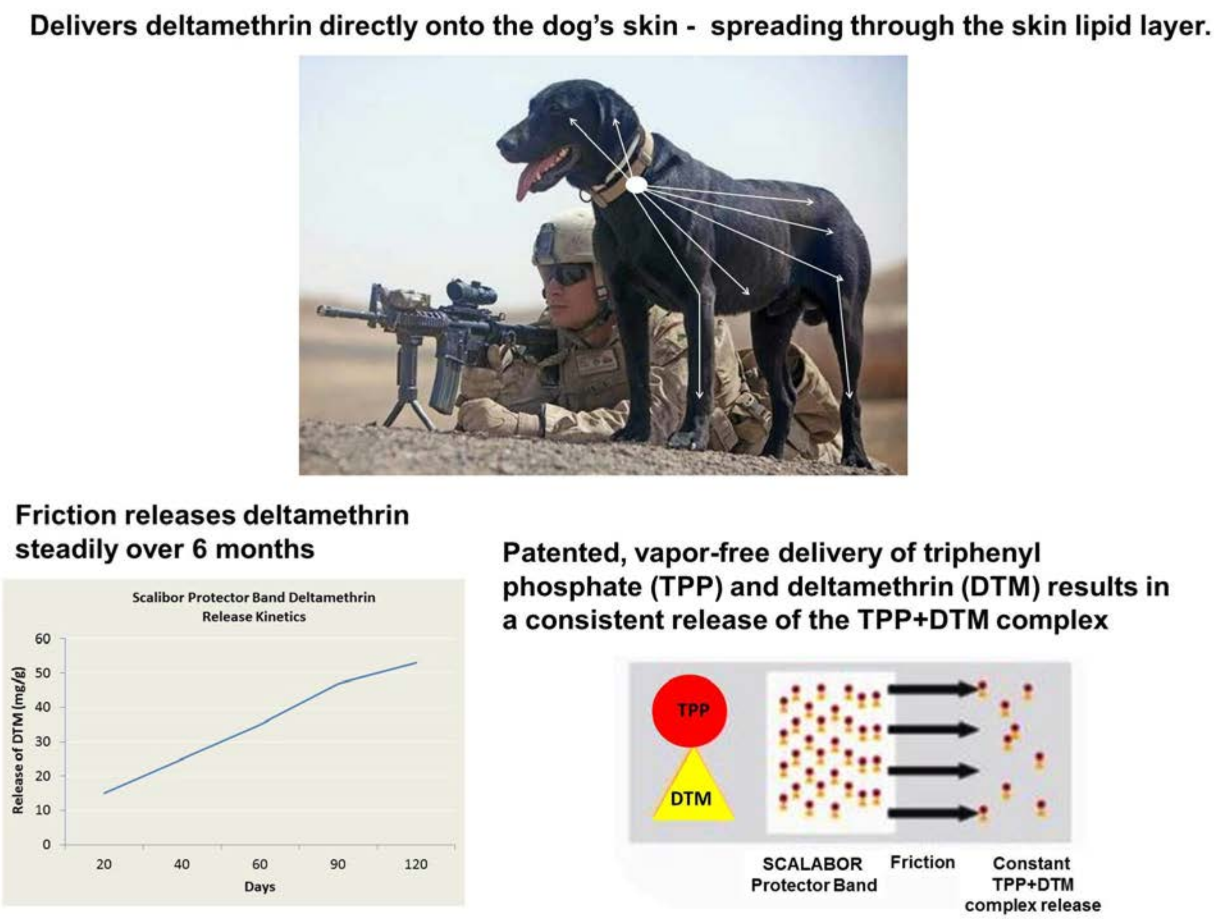


Fig. 6-7. Mode of action for Scalibor® Protector Band. Schematic adapted from manufacturer’s advertising.

6-12. Integration of Multiple Vector Control Techniques

Elimination of sand flies during military operations is an unrealistic goal; however, combining the vector control techniques outlined above, as part of integrated vector management (WHO, 2011), will limit sand fly populations, thereby reducing the risk of disease transmission and the incidence of nuisance sand fly populations. Careful and routine sand fly surveillance should be used to effectively target adulticide efforts, especially aerosol measures. The sand fly control program should combine residual and aerosol adulticide measures, but also include aggressive social engineering campaigns to convince personnel to seal their living and working areas, use PPM and PPE, and resist temptations to expose skin surfaces despite hot conditions.

SECTION 7. POINTS OF CONTACT

7-1 Sources of Assistance for Vector/Pest Identification and Information

- U.S. Army, Walter Reed Biosystematics Unit (WRBU) - Expert insect identification; morphological keys, relevant literature, species distributions by country, high-resolution photographs and information on biomedical importance; assistance with morphological and molecular identification, data management and VectorMap [http://www.wrbu.org/].
- USAF School of Aerospace Medicine Department of Public Health (USAFSAM/AE) - Medical consultants, wide ranging experience in many areas.
- USAFE Command Entomologist - Civil Engineering and medical entomology responsibilities.
- Medical Entomology Services, Detachment 3, USAF School of Aerospace Medicine, Kadena AB, Japan - Medical entomology, pest management consulting and other responsibilities.
- US Army Public Health Command (PHC), http://phc.amedd.army.mil
    - Army Institute of Public Health, Aberdeen Proving Ground, Maryland
    - US Army Public Health Command Region (PHCR) – Europe
    - PHCR-Pacific
    - PHCR-North
    - PHCR-South
    - PHCR-West
- U.S. Army Preventive Medicine Detachments - Entomologists are present at most Preventive Medicine Detachments.
- U. S. Navy - Navy Entomology Center of Excellence (NECE), Naval Medical Research Units (NAMRUs), and Navy Environmental Preventive Medicine Units (NEPMUs) can provide expertise and supplies.
- Armed Forces Pest Management Board (AFPMB) - Information, coordination; Contingency Liaison Officer will answer inquiries, assist in solving problems, coordinate support.
- AFPMB Information Services Division - A valuable information source that will refer issues to appropriate authority for support.
- Local Entomologists and Public Health Support - Most countries have public health personnel (usually government officials) and many have medical/veterinary entomologists (more often at universities and institutes).

- European Centre of Disease Prevention and Control (ECDC), http://www.ecdc.europa.eu/en/healthtopics/vectors/sanflies/Pages/sandflies.aspx
- U.S. Department of Agriculture, Agricultural Research Service, Center for Medical, Agricultural and Veterinary Entomology, Mosquito and Fly Research Unit (USDA-ARS CMAVE MFRU; http://www.ars.usda.gov/main/site_main.htm?modecode=66-15-10-00) can provide information on sand fly control for different ecological regions.

7.2 Biosystematics Resources

A list of keys to the genera, subgenera and species of phlebotomine sand flies, in addition to other systematics resources, can be found at the Walter Reed Biosystematics Unit website (http://www.wrbu.org/VecIDResourcesSF.html). An introductory sand fly tutorial is available at: http://www.wrbu.org/sf_tut/sf_ad_tax_tut00.html

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SECTION 9. ACRONYMS

AFPMB – Armed Forces Pest Management Board
APHIS/PPQ – Animal and Plant Health Inspection Service/Plant Protection and Quarantine
BGS – BioGents Sentinel trap
CDC – Centers for Disease Control and Prevention
CL – Cutaneous leishmaniasis
DCL – Diffuse cutaneous leishmaniasis
DoD – Department of Defense
HESCO – Hercules Engineering Solutions Consortium, www.hescobarriers.com
MCL – Mucocutaneous leishmaniasis
MGRS – Military Grid Reference System
NSN – National Stock Number, for procurement
PKDL – Post kala-azar dermal leishmaniasis
p. – Page
pp. – Pages
PPE – Personal protective equipment
PPM – Personal protective measures
RED HORSE – Rapid Engineer Deployable Heavy Operational Repair Squadron Engineer
sp. – Species (singular)
spp. – Species (plural)
SSAM – Solid state Army miniature light trap
USDA – United States Department of Agriculture
US EPA – United States Environmental Protection Agency
ULV – Ultra low volume
UV – Ultraviolet
VL – Visceral leishmaniasis
WHO – World Health Organization
WRBU – Walter Reed Biosystematics Unit (WRAIR)